Animal Welfare Information Center

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Congress In Session

by Cynthia Smith

- **H.R. 2977 To amend the Federal Advisory Committee Act to clarify public disclosure requirements that are applicable to the National Academy of Sciences and the National Academy of Public Administration.**

Introduce November 10, 1997, by Steve Horn (R-California), passed both House and Senate and cleared for the White House on November 13, 1997. This act may be cited as the "Federal Advisory Committee Act Amendments of 1997."

(1) The Academy shall determine and provide public notice of the names and brief biographies of individuals that the Academy appoints or intends to appoint to serve on [a] committee. The Academy shall determine and provide a reasonable opportunity for the public to comment on such appointments before they are made or, if the Academy determines

(cont'd p. 25)

ALSO IN THIS ISSUE...

Alternatives to Ascites Production of Monoclonal Antibodies

by
John McArdle, Ph.D.
Alternatives Research & Development Foundation
Eden Prairie, Minnesota

Introduction - History

In 1975 George Kohler and Cesar Milstein (13) published a short paper describing their new method for producing monoclonal antibodies (MAbs). Their work included the observations that: (1) "the manufacture of predefined specific antibodies by means of permanent tissue culture cell lines is of general interest," (2) "such cells can be grown in vitro in massive cultures," and (3) "such cultures could be valuable for medical and industrial use." These comments were prophetic, since their new alternative technique was subsequently adopted in virtually every field of biomedical research and biotechnology and in many areas of clinical diagnosis and therapy. So important was this approach to antibody production that Kohler and Milstein received the 1984 Nobel Prize for their discovery.

Although the original research was principally an in vitro technique, it was also apparent that monoclonal antibodies could be produced by injecting the hybridoma cells into the abdominal cavities of different species of rodents. This was the initial use of the ascites method. Since these MAbs were easily made in any laboratory and the ascites process, widely viewed as both simple and inexpensive, was introduced early, its use rapidly expanded. Unfortunately, the original possibility of MAb production replacing uses of laboratory animals was and often continues to be overlooked or ignored. In the decades that followed the original discovery, tens of millions of animals suffered and died despite the availability of more humane alternatives.

During this same period of time the appropriateness of using the ascites method was increasingly being questioned in Europe. Milstein noted that "in later years, both on practical and humane grounds, I became concerned with the use of ascitic fluids" (personal communication, 1997). As new in vitro alternative techniques were developed and validated, it became more difficult to justify the suffering associated with the use of ascites. It simply was not possible to humanely produce MAbs using animal-based procedures.

In 1989, The Netherlands government introduced a Code of Practice for the Production of Monoclonal Antibodies (1), which provided detailed descriptions of the veterinary problems and pathophysiology associated with the ascites process and placed restrictions on its use. The resulting increased humane awareness among Dutch researchers provided further encouragement for adoption of in vitro approaches to MAb production. In 1995, a symposium held in Bilthoven, The Netherlands, concluded that progress in development of such alternatives (both in efficacy and cost) was sufficient that the use of ascites could no longer be justified. The resulting prohibition of animal-based MAb production caused no serious difficulties within the Dutch biomedical re-

search community. Despite initial academic resistance, bans on ascites in Germany and Switzerland experienced similar results, as did the restrictions placed on ascites use in Sweden and the United Kingdom.

By 1996 in vitro production of MAbs was the method of choice in Europe for commercial concerns and others needing large quantities and on the increase for the smaller-scale needs of individual researchers. This latter group made up about 60 percent of European MAb users, primarily for basic research and some diagnostic procedures. Their MAb needs were often met using the ascites technique. A similar situation exists in the United States.

Scientist representatives from several member states of the European Union met in October 1996 at the European Center for the Validation of Alternative Methods to discuss the current status of in vitro and in vivo methods of monoclonal antibody production. After careful consideration of the different types of research and commercial needs for MAbs and the available production options, they concluded that "for all levels of MAb production; there are one or more in vitro methods which are not only scientifically acceptable, but are also reasonably and practically available; and as a consequence, in vivo production can no longer be justified and should cease." (15) The group further called for a Europe-wide prohibition on the routine use of ascites methods of MAb production. In Europe the trend is toward adoption of in vitro alternatives at all stages of the MAb process.

There are three principal steps in production of monoclonal antibodies: 1) immunization, 2) hybridoma formation and 3) MAb production. Each has its own potential for adoption of alternative methods. Although currently done primarily as an in vivo procedure using a few animals, it is possible, especially with human MAbs, to conduct the immunization process entirely in cell cultures. Some technical difficulties remain to be solved before this becomes a routine non-animal-based procedure.

Formation of the hybridoma cells has always been an in vitro technique. However, final production of the monoclonal antibodies involves use of either the ascites or in vitro alternative approaches. The present review briefly focuses on this last step, with an emphasis on the availability of multiple alternative MAb production techniques, suitable for the small-to-medium-scale research and commercial laboratories.

Pros And Cons: Ascites vs. In Vitro

Support for continued use of ascites methods are usually based on claims of: (1) more rapid production and high yields of highly concentrated monoclonal antibodies; (2) minimal requirements for materials, labor, and technical expertise; (3) most hybridomas will grow in mice; (4) relatively less expensive; and (5) either an inherit resistance to or lack of familiarity with in vitro methods.

Experience in European laboratories strongly suggests that "these arguments are increasingly being challenged by documents showing that, depending on the amount and concentration of MAbs needed, in vitro production methods are equally well suited"as ascites (Hendriksen, personal communication). With continually increasing expenses associated with the care of laboratory animals and decreasing prices associated with either the newer in vitro methods or decreased production costs for existing in vitro equipment, the cost differential between animal- and non-animal-based methods is rapidly disappearing.

In contrast with in vivo methods, in vitro approaches to monoclonal antibody production have many positive attributes. In general, MAbs derived from in vitro alternatives express immunoreactivity in ranges of 90 to 95 percent regardless of the method used. This is significantly higher than that with MAbs produced by ascites. Similarly, in vitro cultures rarely fail (3 percent or less), while a much higher percentage of ascitic mice do not produce antibodies. Further, the quality of in vitro MAbs is equal to or better than that derived from in vivo methods.

Glycosylation has been raised as an issue with in vitro methods for producing Mabs. (*Ed. note: Glycosylation can influence the antigen-binding capacity, the resistance of an antibody to proteolysis, and other biologically important processes.*) However, glycosylation patterns are more easily regulated in vitro, since with ascites, they can vary between each individual animal. The ECVAM committee concluded that "there are no reasonable arguments based on antibody glycosylation which support the use of in vivo methods." (15).

Ascites is also subject to criticism on both technical and humane criteria. The major disadvantages of animal-based methods include: (1) association with severe cruelty and suffering to the animals; (2) ascitic fluids may be contaminated with rodent plasma proteins, immunoglobulins (reducing its immunoreactivity), infectious agents and bioreactive cytokines; (3) the need for extensive animal facilities, associated support services with each of the individual animals requiring daily monitoring 7 days a week; (4) some hybridomas (for example, human) are difficult to grow in rodents; (5) rodents only produce MAbs for a few days; (6) from 60 to 80 percent of mice may not produce ascites due to premature death, development of solid tumors, or failure to establish in vivo hybridoma growth (14); and (7) individual batches of ascites may vary significantly in quality and quantity.

The most compelling arguments against in vivo production of

Figure 1. Mouse showing swollen abdomen typical of ascites

(Antibodies cont'd p. 15)

A Whole-Animal Alternative Model for Pain Research

by
Craig W. Stevens, Ph.D.
Department of Pharmacology and Physiology
Oklahoma State University-College of Osteopathic Medicine, Tulsa, Oklahoma

Alternative models for biomedical research seek to address refinement, reduction and/ or replacement of existing animal models for reasons of ethical considerations. Alternative models must also be founded in sound scientific rationale and able to compete for scarce research funds. This brief review describes a unique alternative model using live amphibians for research into opioid analgesia and more generally for pain research.

The ethical basis for an amphibian model stems from a comparative neurological approach to the study of pain and analgesia, which will be described briefly below. (For simplicity, the terms pain and analgesia are applied to non-human animals; some would prefer the more precise terms nociception and antinociception.) At present, we have an active research program using the Northern grass frog, *Rana pipiens,* to investigate the analgesic actions of opioid [6, 10, 15-18] and alpha-adrenergic drugs [2, 13]; morphine tolerance [14]; and stress-induced analgesia mediated by endogenous opioid peptides (endorphins) and the action of enkephalinase-inhibitors [19]. However, the focus of this review is on the ethical and scientific aspects underlying development of the amphibian model, rather than a discussion of our research results. For a more exhaustive review of nonmammalian models for pain research, see elsewhere [12].

Figure 1. The Northern grass frog, *Rana pipiens.*

Why Do Pain Research?

There is no question that use of mammalian models for pain and analgesia research has led to tremendous advances in the understanding of nociceptive transmission, the actions of analgesic drugs, and the function of endogenous opioid systems. Over the past two decades, advances from the biomedical research community have been translated into effective therapeutic interventions for millions of patients suffering from acute and chronic pain syndromes. However, there are a number of pain-related disorders that remain refractory to successful clinical treatment, such as neuropathic pain, and complications of current pharmacotherapy, such as opioid tolerance and dependence, warrant continued research into the basic mechanisms of pain and analgesia using animal models. Additionally, there is an ongoing need for use of animal models in efficacy and safety testing of new analgesics in the pharmaceutical industry.

Special Nature of Animal Use in Pain Research

Unlike most animals used in biomedical research, pain and analgesia researchers use whole behaving animals so that anesthetics or analgesics cannot be administered. As we have no access into the sensorium of an experimental animal, a behavioral test for measuring analgesia must be used. Most analgesic tests used in mammals are self-limiting such that the animal responds to the noxious stimulus and the stimulus is terminated (stimulus-control by the animal). For example, in the rodent tail-flick test, the behavioral response of a mouse or a rat is observed following the presentation of a thermal stimulus and analgesia is measured by the time it takes for the rodent to flick its tail off a projector lamp. The hot plate test entails placing a rat or mouse on a heated surface, usually about 55^{o} C, then measuring the time it takes for the animal to jump or lick its hindpaws. In both cases, the latency to the endpoint is taken before drug or experimental treatment and again at various times after treatment. Other acute analgesic tests include the paw-pressure test and the paw withdrawal from a focused heat source. This last analgesic test is often used in studies of chronic pain in which one hindpaw will be injected with a pro-inflammatory substance (for example, formalin) and the contralateral side will be used as a control. Finally, these types of studies are also done using cats, dogs, and primates, although to a much lesser extent.

Over the past 40 years, thousands of analgesic drugs and treatments have been tested using mammalian models in this way. More recently, the development and popularity of a number of chronic pain models in mammals raises additional ethical issues as there is the possibility of persistent pain in mammals without the ability to terminate the noxious stimulus. The duration of potential pain and its escapability are important ethical considerations for researchers and are included in the guidelines for use of animals in pain research [23].

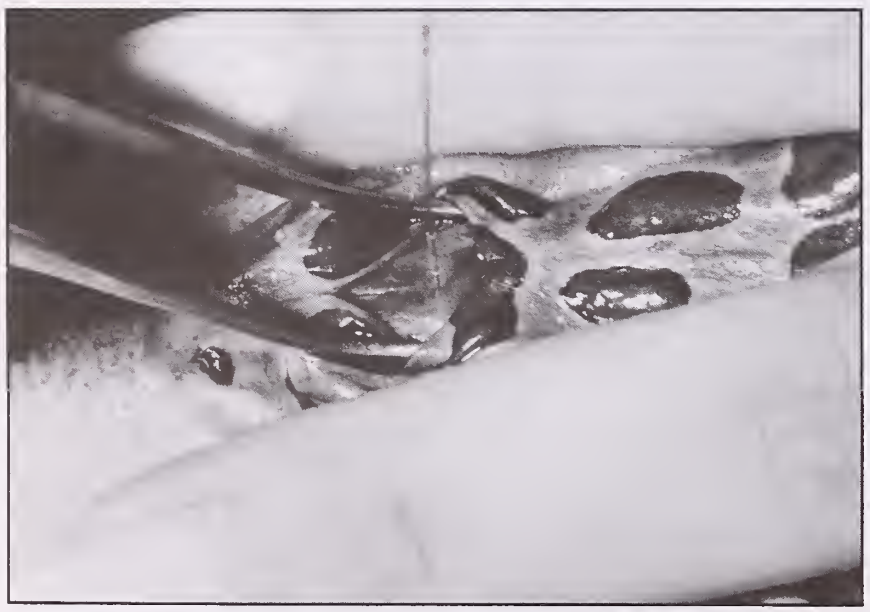

Figure 2. Routes of drug administration in frogs. Top, systemic administration into the dorsal lymph sac. Middle, spinal administration by direct percutaneous intraspinal injection (i.s.). Bottom, supraspinal administration by injector placed in the third ventricle of the brain (i.c.v.). See text for further details.

A Case for Comparative Substitution Using Amphibians

Ethical considerations. Comparative substitution was defined by Russell and Burch as a replacement alternative that substitutes use of a phylogenetically higher species with a lower one, or perhaps better stated, a later-evolved vertebrate with an earlier-evolved one [7]. An appreciation of the phylogenetic classes of animals and differences between species from one class to another is essential for a rational exploration of animal welfare and ethical issues for biomedical research. Even if there is a capacity for pain as we know it in nonhuman animals, there is good reason to suspect that this "pain potential" is correlated with phylogeny. This is an important ethical consideration, as it has been suggested that "it could be morally appropriate to select animals for scientific use based on their capacities for more or less negative experiences." [3] As previously stated, there is less certainty about the capacity of a species to experience pain as we move from humans to other mammals to lower vertebrates [4]. However, a sentience scale can be construed that parallels evolution, and differences in pain capacity between classes of animals may be supported as "the sentience level of an animal is intimately related to its ability to perceive pain." [4] Adding scientific evidence from comparative neurology may also support a gradation of the capacity for pain among vertebrates.

Comparative neurology of pain. Comparing amphibian and mammalian brains, there are significant differences in both the discriminative and affective pathways of pain transmission [9, 11]. There is no thalamus-to-cortex connection in the frog because a cerebral cortex does not appear until class Reptilia. Even this primordial cortex in reptiles is scant and lacking the complex laminar structure seen in mammals. Amphibians simply have a brain without a cerebral cortex. The phylogeny of the medial pathway correlated with motivational-affective aspects of pain is similar whereby in amphibians the most rostral projection reaches to a diffuse olfactory area with little organization of neurons. In mammals, the most rostral target of this pathway is the highly-organized limbic cortex. Again, the beginning of even a rough laminar organization of the limbic area does not appear until class *Reptilia*. The amphibian brain does not contain a limbic cortex. Cortical tissue, whether in limbic or cerebral regions, is a highly complex and laminated structure which is a relatively recent development in the evolution of the nervous system. We know from human experience, that decreasing the activity of cortical neurons by anesthesia or surgical lesion results in a loss of the full appreciation of pain [22]. Recent studies using positron imaging techniques also show specific areas of the cortex activated by noxious stimuli in awake humans [20]. For these reasons, there is widespread agreement among various scientific organizations that an intact cortex is needed for the appreciation of pain [1, 8, 21]. It is likely that amphibians, without either cerebral or limbic cortices, have a vastly diminished potential for the appreciation of pain.

Studies of Opioid Analgesia Using Amphibians

Pezalla first described a method to assess the nociceptive threshold (NT) in frogs using the acetic acid test [5]. The acetic acid test (AAT) to determine the nociceptive threshold in frogs consists of eleven concentrations of acetic acid serial-

ly diluted from glacial acetic acid. Nociceptive testing is done by placing, with a Pasteur pipette, a single drop of acid on the dorsal surface of the frog's thigh. Testing begins with the lowest concentration and proceeds with increasing concentrations until the NT is reached. The NT is defined as the lowest concentration of acid that causes the frog to vigorously wipe the treated leg. To prevent tissue damage, the acetic acid is immediately rinsed off with a gentle stream of distilled water once the animal responds, or after 5 seconds if the animal fails to respond. Our results show that using the AAT in amphibians gives a rank order of the relative analgesic potency highly correlated with that found in rodent models after systemic and spinal administration of *mu*-, *delta*-, and *kappa*-selective opioids [10, 15]. These results suggest that the analgesic action of opioid agents in amphibians is predictive of the analgesic effects of opioids seen in humans and other mammals. This fundamental finding also supports use of an amphibian model for the high throughput testing of potential analgesic agents, where lower cost may be an advantage.

Conclusions

Comparative substitution is a moderate approach to animal replacement alternatives as a whole animal is still used rather than cells or tissue, but amphibians may have considerably less potential for pain than mammalian models currently in use. This is important as whole animals must be used for pain and analgesia research ("cells do not feel pain").

In general, groups promoting the 3Rs of animal welfare for biomedical research have overlooked the immediate welfare gains that may be possible by using comparative substitution as an alternative model. Finally, these studies provide novel data on the efficacy of opioid analgesics in amphibians that may be important for the veterinarian treating amphibians in the clinic.

Acknowledgments

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The author may be reached at Craig W. Stevens, Ph.D., Dept. of Pharmacology/Physiology, OSU-COM, 1111 West 17th Street, Tulsa, OK 74107-1898 USA, phone: (918) 561-8234, fax: (918) 561-8412, email: scraig@okway.okstate.edu

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Reducing Animal Research at the U.S. Army Medical Research Institute of Chemical Defense

by
Major Randall E. Greenfield, Chief, Comparative Medicine Division; Cindy A. Kronman, Research Operations Division;
Colonel David H. Moore, Deputy Commander, U.S. Army Medical Research Institute of Chemical Defense
3100 Ricketts Point Road, Aberdeen Proving Ground, Maryland

The U.S. Army Medical Research Institute of Chemical Defense (USAMRICD) at Aberdeen Proving Ground, Maryland, is the lead laboratory for executing the Department of Defense's medical chemical defense research program. The USAMRICD employs 141 permanent civilians and 55 military officers, and supplements this staff with National Research Council fellows, interns from the Oak Ridge Institute for Science and Education (ORISE) postgraduate internship program, and contract personnel. There are approximately 42 principal investigators, who carry out a research budget of over $16 million.

Their mission is to develop medical countermeasures against exposure to chemical warfare agents as well as against agents of biological origin. While accomplishment of this research mission necessarily requires in vivo studies, in the last 11 years, scientists at the USAMRICD have reduced the number of animals used in their research protocols by 92 percent.

These efforts to reduce use of animals in research are part of a larger program initiated by the Department of Defense, and more specifically by the U.S. Army Medical Research and Matériel Command (USAMRMC), of which the USAMRICD is a subordinate unit. In 1992, the U.S. House of Representatives Committee on the Armed Services issued a report on use of animals in DoD military experiments. The resulting action was the 1993 National Defense Authorization Act, requiring DoD to submit a comprehensive annual report on animal cost and use as well as in depth profiles of animal research and initiatives to promote alternatives to reduce, refine, and replace (the 3Rs). In 1995, DoD issued directives and policies that updated and redefined its requirements for the care and use of animals in DoD-sponsored programs. In adopting the 3Rs, the DoD also added a fourth, responsibility.

In 1993, the USAMRMC developed a management-by-objective program based on science and technology objectives, or STOs, with specific tasks under each objective. Among the major objectives established by the USAMRMC was one to develop reduction, refinement, and replacement strategies for use of animals in research. The goal was to develop technologies that would incrementally reduce reliance on animal and human subject research and improve the experimental conditions using animals. Current objectives are to reduce reliance on animals in research by 25 percent, by fiscal year 1999, using data from fiscal year 1991 as a baseline, and to introduce a minimum of one improvement per year in experimental protocols using animals.

The USAMRICD – AAALAC-accredited since 1984 and with an established animal care and use committee overseeing animal research since the late 1970s – was already in compliance with much of the Department of Defense direc-

Figure 1. Cultured normal HeLa cells. This cell line is derived from a human cervical epithelial tumor cell.

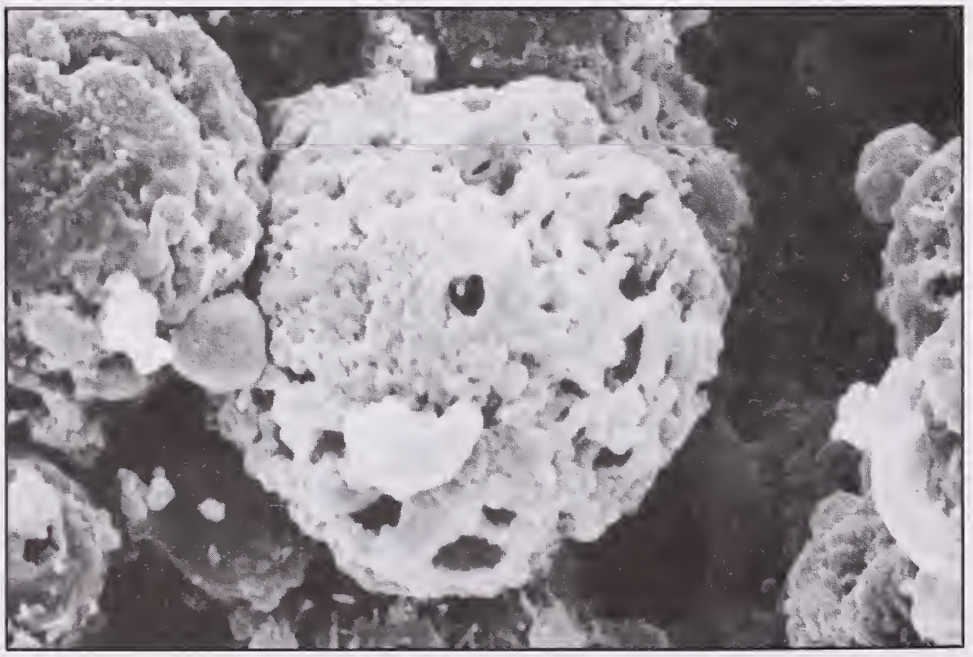

Figure 1a. Cultured HeLa cells 24 hours after exposure to sulfur mustard. Note the loss of microvilli and multiple perforations and invaginations of the plasma membrane.

tive when it was issued in 1995 and well on its way to meeting the goals of its Command's research objectives. Principal investigators at the USAMRICD have taken advantage of emerging technologies in design of molecular modeling software and in development and maintenance of cell culture models to meet the animal reduction goals of the USAMRMC. In addition, scientists, where possible, have adopted the use of less sentient animal species.

Computer modeling of the molecular structure of nerve agents, physiological enzymes, and neurotransmitters is used to predict and eventually determine how these chemical compounds interact at the molecular level. A test compound that inhibits acetylcholinesterase (AChE) aging has also been studied via computer modeling to determine the specific molecular events that occur at the peripheral anionic site of AChE that subsequently prevent a potent AChE inhibitor, such as a nerve agent, from irreversibly binding to the acetylcholinesterase molecule. With this knowledge, the most likely candidates for chemical intervention in the prevention or treatment of nerve agent exposure can be determined.

Currently, 17 cell lines or cell cultures are used in research at the USAMRICD. While they are used in research studies across the Institute's program, they have particularly led to a reduction of animal usage in the research programs on blistering chemical warfare agents. In efforts to evaluate the effects of blister agents, or vesicants, particularly sulfur mustard, and to develop medical countermeasures against such agents, alternatives to animals have proven particularly useful. Several cell line or culture systems, such as peripheral blood lymphocytes, human epidermal keratinocytes (figs. 1 and 1a), and the HeLa, a human epithelial tumor line (figs. 2 and 2a), have been adapted and developed by scientists at the USAMRICD into valuable models for studying vesicant injury. In addition, studies have used a commercial human skin equivalent model and skin biopsies from the Cooperative Human Tissue Network. The USAMRICD has also developed the technology to process and generate a human epidermal model, which possesses typical structural components of human epidermis in vivo to include hemidesmosomes, anchoring filaments, and elements of a true basement membrane. The use of these alternative models has led especially to a decrease in the number of rodents required for these studies.

The USAMRICD also promotes the use of less sentient or less regulated animal species. Researchers at the USAMRICD use the *Aplysia californica,* a large naked marine mollusk or gastropod with anterior sensory tentacles, commonly referred to as the sea hare or sea slug. The *Aplysia californica* possesses rather large and discrete neural ganglia, unique neural anatomy that make these invertebrate animals an excellent and widely used model for neural transmission and neurotoxin research. The USAMRICD acknowledges that in certain sulfur mustard experimental protocols, SKH-1 hairless mice are an appropriate substitute for the hairless guinea pig. The main histological feature of skin lesions produced in a SKH-1 hairless mouse following exposure to sulfur mustard is the formation of microblisters at the dermal-epidermal junction. Similar microblisters also occur in sulfur mustard lesions of hairless guinea pigs. Whereas laboratory mice are not subject to the Animal Welfare Act, provisions for their care and use are contained in the Guide for the Care and Use of Laboratory Animals.

Through these alternatives to animal research initiatives, the USAMRICD is 3 years ahead of schedule and has already exceeded the 25 percent reduction in use of animals required by the USAMRMC's research objective. From fiscal year 1991 to fiscal year 1996, the Institute's reliance on animals in its research dropped by 68 percent. The most dramatic drop was in use of rodents in research; 90 percent fewer rodents were used in research protocols in 1996 than were used in 1985. The number of publications resulting from research at the USAMRICD, however, has remained high and even increased slightly over the last several years. Approximately 34 percent of the Institute's 1996 publications were a result of research in which no animals or less sentient animal species were used. ■

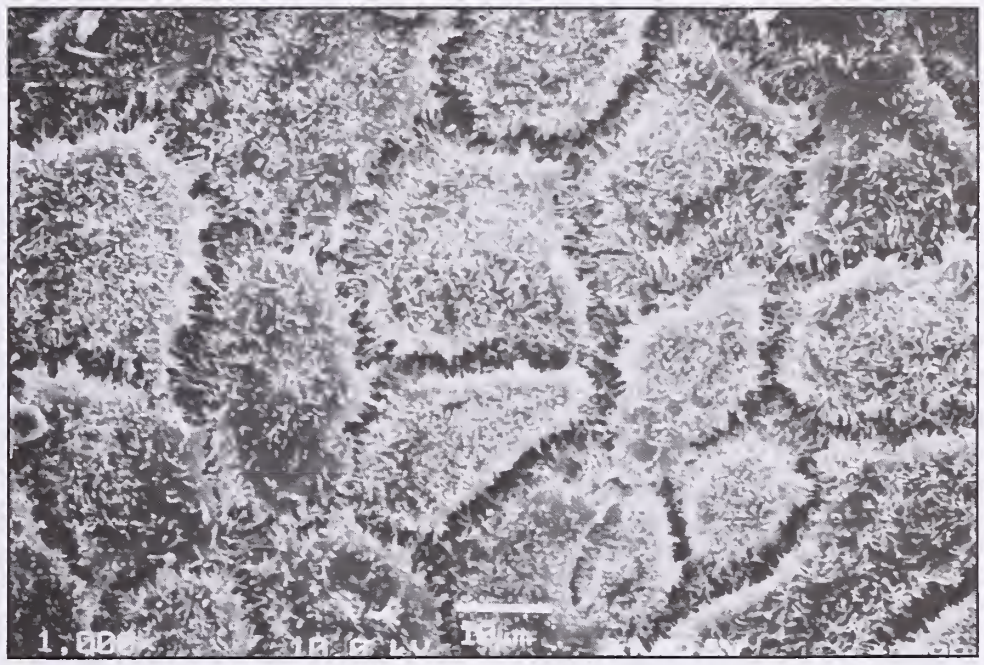

Figure 2. Cultured normal human epidermal keratinocytes.

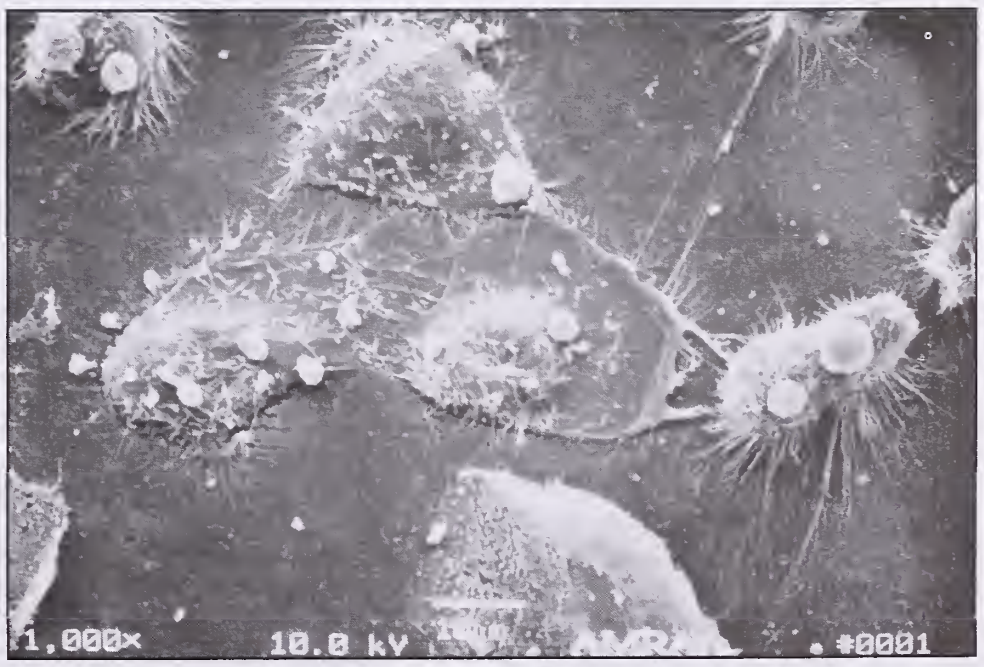

Figure 2a. Cultured normal epidermal keratinocytes 6 hours after exposure to sulfur mustard. Note the loss of microvilli, focal plasma membrane blebbing, and cellular detachment/loss subsequent to cell death.

Assessment and Alleviation of Post-Operative Pain

by
Paul Flecknell
University of Newcastle, Comparative Biology Centre, Medical School, Framlington Place, Newcastle-upon-Tyne, United Kingdom

Abstracted by Paul Flecknell from his book *Laboratory Animal Anaesthesia* 2nd Edition, 1996, and presented at the CALAS/ACTAL Convention in Prince Edward Isle, Canada

This article originally appeared in the CALAS/ACSAL Newsletter Vol 30 #5 October 1996. It is reprinted with the permission of the Canadian Association for Laboratory Animal Science/L'-Association Canadienne pour la Science des Animaux de Laboratoire.

The effective alleviation of post-operative pain in laboratory animals should be considered an important goal in all research establishments. Despite the emphasis given to humane treatment of laboratory animals in the national legislation of many countries, analgesia may still not be administered routinely in the post-operative period. This omission is particularly common when the animals concerned are small rodents. When analgesics are administered, assessment of methods of pain recognition or severity may account in part for the relatively infrequent use of analgesics in animals, in comparison to their use in man. This is not meant to imply that veterinary surgeons and others involved in animal care are incapable of recognizing that an animal is in pain, but preconcepti[illegible] animal pain may limit the va[illegible]ay assessment of its severity (see below).

Although we would wish to alleviate pain because of concerns for animal welfare, a number of counter-arguments have been advanced to justify withholding analgesics:

Alleviation of post-operative pain will result in the animal injuring itself. Provided that surgery has been carried out competently, administration of analgesics, which allow resumption of normal activity, rarely results in problems associated with the removal of pain's protective function. Claims that analgesic administration results in skin suture removal are unsubstantiated, and contrary to findings in our laboratory. In certain circumstances, for example after major orthopaedic surgery, additional measures to protect and support the operative site may be required, but this is preferable to allowing the animal to experience unrelieved pain. All that is required in these circumstances is to temporarily reduce the animal's cage or pen size, or to provide additional external fixation or support for the wound. It must be emphasized that these measures are very rarely necessary, and in our institute, administration of analgesics to laboratory animals after a wide variety of surgical procedures has not resulted in any adverse clinical effects.

Analgesic drugs have undesirable side-effects such as respiratory depression. The side-effects of opiates in animals are generally less marked than in humans and should rarely be a significant consideration when planning a post-operative care regimen.

We don't know the appropriate dose rates and dosage regimens. This is primarily a problem of poor dissemination of existing information. Virtually every available analgesic drug has undergone extensive testing in animals. Dose rates are therefore available for a range of drugs in many common laboratory species (17, 36). It is occasionally difficult to extrapolate available dose rates from one species to another and to translate dose rates that are effective in experimental analgesiometry into dose rates which are appropriate for clinical use. Nevertheless, in most instances, a reasonable guide as to a suitable and safe dose rate can be obtained.

Pain relieving drugs might adversely affect the results of an experiment. Although there will be occasions when the use of one or another type of analgesic is contra-indicated, it is extremely unlikely that there will be no suitable analgesic that could be administered. More usually, the reluctance to administer analgesics is based upon the misconceived idea that the use of any additional medication in an experimental animal is undesirable. The influence of analgesic administration in a research protocol should be considered in the context of the overall response of the animal to anaesthesia and surgery. The responses to surgical stress may overshadow any possible adverse interactions associated with analgesic administration. An additional consideration is that many arrangements for intraoperative care fail to control variables such as body temperature, respiratory function and blood pressure. It seems illogical to assume that changes in the function of the cardiovascular or respiratory systems are unimportant, but that administration of an analgesic will be of overriding significance. It should be considered an ethical responsibility of a research worker to provide a reasoned, scientific justification if analgesic drugs are to be withheld. It is also important to realize that the presence of pain can produce a range of undesirable physiological changes, which may radically alter the rate of recovery from surgical procedures (28).

Progress In Pain Assessment

When debating the nature of pain in animals, considerable parallels can be drawn with the situation in human infants. In adult humans, the ability to provide direct verbal communication, complete pain questionnaires or scoring systems, or to directly manage analgesic dosage using patient controlled analgesia systems allows reasonably reliable estimates to be made of the degree of pain and the efficacy of pain control. In young human infants, written and verbal communication is not possible, nevertheless, extrapolation from adult humans, coupled with objective demonstrations of the adverse effects of surgical stress, has led to a huge increase in interest in providing pain relief to these patients (2, 41).

The approaches used in human infants can provide a framework for animal pain assessment. The most widely used techniques have been pain scor-

ing systems based upon criteria such as crying, facial expression, posture and behaviour (42). This type of approach was advocated as a means of assessing pain and distress in animals (43). This paper influenced a large number of other groups, who modified the original hypothesis, but retained the central notion of identifying pain specific behaviours, and rating them in some way (3, 18, 33). Surprisingly, progress in validating this hypothesis has been remarkably slow. An early report (35) indicated that the technique could be applied successfully, but the few subsequent published data are less encouraging. Particular problems noted were considerable between observer variation and the poor predictive value of certain of the parameters scored (5, 6). The between observer variation is not unexpected, and parallels problems recognized in human pain scoring. It appears that if the number of observers is restricted, and the criteria used was carefully selected, reasonable agreement can be achieved (49).

The basic methodology–selecting clinical signs which might be due to pain–has been used to provide pain-scoring systems in veterinary clinical patients. Attempts at scoring have either used descriptive ratings converted to numerical scores to allow statistical analysis, or have used visual analogue scoring systems (VAS) (45, 46, 48, 49, 53). A problem with many of these studies is the difficulty associated with scoring of animal behaviour in a relatively brief period. If it is believed that behavioural responses can indicate pain, and hence the efficacy of analgesia, then more detailed assessments are likely to be required. Support for the value of behavioural observations is provided by studies of the effects of tail docking and castration in lambs (54) and castration in piglets (40).

In laboratory animals, a number of different approaches have been used to assess pain or distress. The most extensive studies have been undertaken to investigate chronic pain, for example, those by Colpaert et al (8, 9, 10, 11), using an adjuvant arthritis model in the rat. Body weight, minute volume of respiration, mobility, vocalizations, specific behaviours and self-administration of analgesics were all considered as indices of pain. When discussing the results of these investigations, the authors concluded that all of the parameters responded to the same stimulus, and that the most reasonable explanation was that they were influenced by the presence of pain (9). Motor behaviour changes have been suggested as indices of pain (7, 55) and loss of appetite and reduction in body weight have been noted in rodents post-operatively (23, 24, 55). Recently, these variables have been studied in rats as potential means of assessing the degree of post-operative pain, and comparing the efficacy of different analgesic regimens (20, 21, 37, 38). As with other pain assessment techniques in animals, these assumed that if a change to a variable occurred after a procedure that would cause pain in man, then the change may be related to pain in the animal. If administration of an analgesic reverses the changes associated with the procedure, this supports the hypothesis that the changes were, at least in part, pain related. Clearly it is important to establish that the analgesic did not have non-specific effects in normal animals that would influence the variable studied. This is a somewhat circular argument, since it is simply stating that indices of pain are those indices that are normalized by administration of analgesic drugs. Although efficacy of these analgesics in reducing peripheral input in animals is well-established, (1, 13, 31, 52), their effects on clinical pain are only validated in humans.

The uncertainty surrounding pain scoring could be circumvented if some independent validation method were available. In man, a series of objective criteria have been proposed to assess pain. These have included pulse rate, skin conductance and resistance, blood pressure and skin temperature. In addition, biochemical and endocrine parameters, such as blood corticosterone or cortisone concentrations or catecholamine concentrations, have been proposed as indicating pain. A major problem in interpreting the significance of these changes is the influence of surgery and anaesthesia, which markedly alter many of these variables, even in patients which are pain free (29). The surgical stress response occurs in all patients, and although it can be reduced by intra-operative use of

Drug	Mouse	Rat	Guinea Pig	Rabbit	Ferret
Aspirin	120mg/kg po	100 mg/kg po	87 mg/kg po	100 mg/kg po	200 mg/kg po
Carprofen		5 mg/kg sc		1.5 mg/kg po twice daily	
Diclofenac	8 mg/kg po	10 mg/kg po	2.1 mg/kg po		
Flunixin	2.5 mg/kg sc, im ?12 hourly	2.5 mg/kg sc, im ? 12 hourly		1.1 mg/kg sc, im ? 12 hourly	0.5-2 mg/kg sc 12-24 hourly
Ibuprofen	30 mg/kg po	15 mg/kg po	10 mg/kg im 4 hourly	10 mg/kg iv 4 hourly	
Indomethacin	1 mg/kg po	2 mg/kg po	8 mg/kg po	12.5 mg/kg po	
Ketoprofen		5 mg/kg po		3 mg/kg im	
Paracetamol (Acetomino-phen)	200 mg/kg po	200 mg/kg po			
Piroxicam	3 mg/kg po	3 mg/kg po	6 mg/kg po		

Table 1. Suggested dose rates for non-steroidal anti-imflammatory drugs. Note that considerable individual and strain variation in response may be encountered, and it is therefore essential to assess the analgesic effects in each animal. Abbreviations: im = intramuscular, iv = intravenous, po = per os (by mouth), sc = subcutaneously, ? = approximate duration.

opioids, it occurs even in patients who receive a high level of post-operative pain control. In man, catecholamine and cortisol responses have shown to be poorly correlated with post-operative pain scores. Use of these variables in animals has the same constraints. Catecholamine rises have been demonstrated in cats (4) and dogs (48), and cortisol response is less following thoracotomy when epidural morphine rather than intravenous morphine is administered (48). However, lack of appropriate controls and influence of surgical stress limit the significance that can be attached to these studies. Despite these reservations, studies such as those of Popilskis et al (48), which correlate both subjective pain scores and endocrine responses, advance a persuasive case of the validity of pain scoring. Nevertheless, the difficulties highlighted by studies in man suggest that biochemical indices are unlikely to provide a reliable objective method of pain assessment in animals.

Pain Relief

Leaving aside the problems of pain assessment, empirical treatment of presumed painful conditions will continue, and it is not unreasonable to assume that analgesic therapies shown to be effective in man are likely to also be effective in animals. Although the assessment of clinical efficacy may not have been completed, studies of novel analgesic compounds and delivery systems in animals have established their safety and efficacy in analgesiometric tests. Analgesics can be broadly divided into two groups, the opioids or narcotic analgesics and the non-steroidal anti-inflammatory drugs (NSAID) such as aspirin. Local anaesthetics can also be used to provide post-operative pain relief by blocking all sensation from the affected area. Suggested dose rates of analgesics are given in tables 1-4.

Clinical Use of Analgesics

A number of clinical problems arise when analgesics are administered to control post-operative pain. The most important problem is the short duration of action of most of the opioid (narcotic) analgesics. Maintenance of effective analgesia with, for example, pethidine, may require repeated administration every 1-3 hours, depending upon the species. Continuation of such a regime overnight can cause practical problems. One method of avoiding this difficulty is to use buprenorphine as the analgesic, since there is good evidence in humans, rodents, rabbits and pigs that it has a duration of action for 6-12 hours (12, 16, 19, 25, 26). In clinical use in a wide range of animal species, it appears to provide effective pain relief to 6-12 hours. Its duration of action in the sheep appears to be considerably less, although still of longer duration than pethidine and morphine (44).

An alternative approach is to adopt the well-established human clinical technique of administering analgesics as a continuous infusion. Infusions of analgesics have the advantage of maintaining effective plasma levels of the analgesic, thus providing continuous pain relief. This is in contrast to intermittent injections, where pain may return before the next dose of analgesic is administered. This technique obviously poses some methodological difficulties in animals, but if an indwelling catheter and harness and swivel apparatus are available, this can be arranged quite simply. In larger species (3-4 kg body weight), a light weight infusion pump can be bandaged directly to the animal and continuous infusion made simply by means of a butterfly type needle anchored subcutaneously or intramuscularly. When analgesics are to be administered by continuous infusion, the infusion rate can be calculated from a knowledge of the pharmacokinetics of the analgesia to be used. If these data are not readily available, an approximation that appears successful in clinical use is as follows: calculate the total dose required over the period of infusion, reduce this by half and set the pump infusion rate accordingly; administer a single, normal dose of the drug as an initial loading dose and start the infusion. The rate can then be adjusted depending upon the animal's responses.

Drug	Pig	Sheep	Primate	Dog	Cat
Aspirin		50-100 mg/kg po 6-12 hourly	20 mg/kg po 6-8 hourly	10-25 mg/kg po 8 hourly	10-25 mg/kg po every 48 hours
Carprofen	2-4 mg/kg iv or sc once daily	?	?	4 mg/kg iv or sc once daily 1-2 mg/kg bid po for 7 days	4 mg/kg iv or sc every 24 hours
Flunixin	1-2 mg/kg iv or sc once daily	2 mg/kg iv or sc once daily	2-4 mg/kg sc once daily	1 mg/kg iv or sc 12 hourly 1 mg/kg po for up to 3 days	1 mg/kg sc daily for up to 5 days
Ibuprofen				10 mg/kg po 24 hourly	
Ketoprofen				2 mg/kg sc, iv, or im once daily for up to 3 days 1 mg/kg po daily for 5 days	1 mg/kg sc once daily for up to 3 days 1 mg/kg po daily for 5 days
Paracetamol (Acetominophen)				15 mg/kg po 6-8 hourly	Contraindicated
Piroxicam				300 μg/kg every 48 hours	

Table 2. Suggested dose rates for non-steroidal anti-inflammatory drugs in laboratory animals. Note that considerable individual and strain variation in response may be encountered, and that it is therefore essential to assess the analgesic effects in each individual animal.

Alternative Routes Of Administration

Attempts to provide both longer periods of pain control and more effective analgesia have led to the development of alternative methods of drug delivery. The majority of these techniques have been developed in man and some have been used successfully in companion animals.

Epidural and Intrathecal Opioids

Epidural and intrathecal opioids have been shown to have a prolonged effect in man, and to provide effective analgesia. In animals, clinical studies and experimental data indicate that the technique can be used in a number of species (14, 15, 45, 46, 48). Although used as a research tool in laboratory species (56), this route of administration has yet to be exploited as a means of controlling post-operative pain. The necessary techniques of epidural or intrathecal injection have been described in the rabbit (27, 30). In larger species such as the cat, dog, sheep, and pig, descriptions of the injection technique can be found in most veterinary anaesthesia texts and a number of other publications (eg., 32, 39).

Oral Administration

The need for repeated injections of analgesics is time consuming and may be distressing to the animal, particularly smaller species which require firm physical restraint for an injection to be given safely and effectively. In addition, the need for repeated injections requires veterinary or other staff to attend the animal overnight. To circumvent this problem, the possibility of incorporating analgesics in food or water has been investigated (31). Long-term analgesia can be produced by this route. Kistler (31) reported that rats demonstrated analgesia for a two week period when buprenorphine was administered continuously in the drinking water. Unfortunately, several practical problems limit the use of this technique. Some animals eat and drink relatively infrequently or may only do so in the dark phase of their photo period. In addition, food and water intake may be depressed following surgery, and this, coupled with wide individual variation in consumption, make routine application of the technique difficult. Finally, the high first-pass liver metabolism of opioids administered by the oral route requires that high dose rates are given, and this can represent a significant cost if all of the animals drinking water or food are medicated.

Administration of small quantities of medicated food does not avoid the need for repeated attendance overnight, but does remove the need for repeated subcutaneous or intramuscular injections in small rodents. Provision of analgesia with buprenorphine in flavoured gelatin, "Buprenorphine Jello"(47), seems to be an effective means of providing post-operative pain relief. In our laboratory, we have noted that rats are initially cautious of jelly pellets, but once one pellet has been consumed, subsequent pellets are eaten as soon as they are offered. It is therefore advisable to commence administering pellets, which do not contain analgesic 2 to 3 days before surgery. After surgery, analgesic containing jelly can be given. The flavoured gelatin used is domestic fruit-flavoured jelly reconstituted at double the recommended strength.

Techniques for administration of food pellets at intervals to experimental animals are well-established, and it would be a relatively simple procedure to introduce an automated means of delivering pellets at appropriate time intervals. The technique could also be used with larger species and need not be restricted to opioids or, indeed, analgesics. Provided that the animal is eating or drinking, small quantities of highly palatable material could be provided at appropriate intervals. Simple timer devices to achieve this are already marketed for delayed feeding of pet dogs and cats.

As mentioned above, the administration of opioids by any route can be associated with the development of respiratory depression. It must be emphasized that this is rarely of clinical significance in animals, unless high doses of pure mu agonists (for example, fentanyl) are used. If respiratory depression occurs, it can be treated by the administration of the opiate antagonist drug, naloxone. Administration of naloxone will also reverse the analgesic effects of the opioid, and it may be preferable to correct the respiratory depression by the use of doxapram. Alternatively, if a mu opioid agonist such as morphine or fentanyl has been used, the respiratory depression can be reversed using nalbuphine or butorphanol, and some analgesia maintained because of the action of these latter two agents at kappa receptors.

Drug	Mouse	Rat	Guinea Pig	Rabbit	Ferret
Buprenorphine	0.05-0.1 mg/kg sc 12 hourly	0.01-0.05 mg/kg sc, iv 8-12 hourly 0.1-0.25 mg/kg po 8-12 hourly	0.05 mg/kg sc 8-12 hourly	0.01-0.05 mg/kg sc, iv 8-12 hourly	0.01-0.03 mg/kg iv, im, sc 8-12 hourly
Butorphanol	1-5 mg/kg sc 4 hourly	2 mg/kg 4 hourly		0.1-0.5 mg/kg iv 4 hourly	0.4 mg/kg im 4-6 hourly
Morphine	2.5 mg/kg sc 2-4 hourly	2.5 mg/kg sc 2-4 hourly	2-5 mg/kg sc, im 4 hourly	2-5 mg/kg sc, im 2-4 hourly	0.5-5 mg/kg sc, im 6 hourly
Nalbuphine	4-8 mg/kg im ? 4 hourly	1-2 mg/kg im 3 hourly	1-2 mg/kg iv, im, ip	1-2 mg/kg iv 4-5 hourly	
Pentazocine	10 mg/kg sc 3-4 hourly	10 mg/kg sc 3-4 hourly		5-10 mg/kg sc, im, iv 4 hourly	
Pethidine (Meperidine)	10-20 mg/kg sc or im 2-3 hourly	10-20 mg/kg sc, im 2-3 hourly	10-20 mg/kg sc, im 2-3 hourly	10-20 mg/kg sc, im 2-3 hourly	5-10 mg/kg sc, im 2-4 hourly

Table 3. Suggested dose rates for opioid analgesics in common laboratory animals. Note that considerable individual and strain variation in response may be encountered, and that it is therefore essential to assess the analgesic effects in each individual animal.

Repeated administration of these agents may be required, and the animal should be observed carefully for several hours to ensure that adequate respiratory function is maintained.

Additional Considerations in Pain Relief

Although the use of analgesic drugs remains the most important technique for reducing post-operative pain, the use of these drugs must be integrated into a total scheme for perioperative care. Pain relief in the immediate recovery period can be provided by including an analgesic drug in any preanaesthetic medication. Alternatively, if a neuroleptanalgesic combination has been used to produce anaesthesia, it can be reversed by the use of buprenorphine,nalbuphine or butorphanol, rather than with naloxone. These agents have been shown not only to reverse the respiratory depressant effects of opioids such as fentanyl but, in contrast to naloxone, to provide effective prolonged analgesia (22, 34, 50).

The expertise of the surgeon can also greatly influence the degree of post-operative pain. Good surgical technique which minimizes tissue trauma and the prevention of tension on suture lines can considerably reduce post-operative pain. The use of bandages to pad and protect traumatized tissue must not be overlooked and forms an essential adjunct to the use of analgesic drugs.

Aside from measures directed towards alleviating or preventing pain, it is important to consider the overall care of the animal and the prevention of distress. Distress is used in this context to describe conditions which are not in themselves painful, but which are unpleasant and which the animal would normally choose to avoid. For example, recovering from anaesthesia on wet, uncomfortable bedding in a cold, unfamiliar environment would be likely to cause distress to many animals. It is essential to consider the methods described for the control of pain in conjunction with the techniques discussed earlier aimed at providing good post-operative care.

Recommendations

It is difficult to make firm recommendations concerning which analgesics to use routinely, and how often to give them, because of the various factors outlined above. Nevertheless, as a general guide, the following techniques are used routinely in the author's research facility.

When carrying out any surgical procedure, buprenorphine is administered either pre-operatively or immediately following induction of anaesthesia, if a volatile anaesthesia is used. If neuroleptanalgesic regimens are used, or mu opioids are given as part of a balanced anaesthetic technique, then administration of buprenorphine is delayed until completion of surgery. If the procedure is relatively minor (for example, jugular or carotid cannulation) then only a single dose of analgesic is administered. In some circumstances a potent nonsteroidal anti-inflammatory drug (NSAID), such as flunixin or carprofen, may be used as an alternative to buprenorphine.

Following more invasive surgical procedures, such as laparotomy, orthopaedic surgery or craniotomy, opioid administration is continued for 24-48 hours. When undertaking major surgery, particularly in larger species when the degree of tissue trauma tends to be greater, analgesic administration may continue for 72 hours. Frequently, the regimen chosen consists of opioids (buprenorphine) in combination with an NSAID for 24-36 hours, followed by NSAID alone for a further 24-36 hours. (See tables 1-4 for suggested dose rates.)

Conclusions

Providing effective post-operative pain relief can have a dramatic effect on the speed with which animals return to normality following surgical procedures. It has been repeatedly demonstrated in humans that the provision of effective analgesia reduces the time taken for post-operative recovery (51). The provision of good post-operative care should be considered essential both because of a concern for the animal's welfare and also because it is good scientific practice.

Paul Flecknell may be contacted at e-mail: p.a.flecknell@newcastle.ac.uk

Drug	Pig	Sheep	Primate	Dog	Cat
Buprenorphine	0.005-0.02 mg/kg im, iv 6-12 hourly	0.005-0.01 mg/kg im, iv 4 hourly	0.005-0.01 mg/kg im, iv 6-12 hourly	0.005-0.02 mg/kg im, iv, sc 6-12 hourly	0.005-0.01 mg/kg iv, sc 8-12 hourly
Butorphanol			0.01 mg/kg iv ?3-4 hourly	0.2-0.4 mg/kg im, sc 3-4 hourly	0.4 mg/kg sc 3-4 hourly
Morphine	0.2-1 mg/kg im ?4 hourly	0.2-0.5 mg/kg im ?4 hourly	1-2 mg/kg im, sc 4 hourly	0.5-5 mg/kg im, sc 4 hourly	0.1 mg/kg sc 4 hourly
Nalbuphine				0.5-2.0 mg/kg im, sc 3-4 hourly	1.5-3.0 mg/kg iv 3 hourly
Oxymorphone				0.05-0.22 mg/kg im, iv, sc 2-4 hourly	0.2 mg/kg iv, sc
Pentazocine	2 mg/kg im, iv 4 hourly		2-5 mg/kg im, iv 4 hourly	2 mg/kg im, iv 4 hourly	
Petidine (Meperidine)	2 mg/kg im, iv 2-4 hourly	2 mg/kg im, iv 2-4 hourly	2-4 mg/kg im, iv 2-4 hourly	10 mg/kg im 2-3 hourly	2-10 mg/kg im, sc 2-3 hourly

Table 4. Suggested dose rates for opioid analgesics in other animals. Note that considerable individual and strain variation in response may be encountered, and it is therefore essential to assess the analgesic effects in each animal.

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Correction

In AWIC Newsletter Vol 8 #2 Summer 1997, the article on page 13–"IACUC: Expedited Review of Animal Use Protocols"– by Richard Crawford, D.V.M., should have stated that each member of an Institutional Animal Care and Use Committee (IACUC) shall be "provided with a list of proposed activities to be reviewed" rather than a copy of the protocol to be reviewed. However, to ensure that informed decisions are being made by members of IACUCs, all members should have the opportunity to review the full animal protocol prior to voting. Also, there is no Federal requirement that the *full* committee review a protocol (regardless of the nature of the pain or distress that animals may experience). However, because those protocols that do contain painful procedures will receive a more thorough review at the full committee level, the USDA encourages this practice. In this way, the IACUC, as an agent of the facility, can more easily assure itself that pain and distress are being kept to a minimum. We apologize for any confusion this may have caused. ■

Antibodies cont'd from p.2

monoclonal antibodies are based on the suffering associated with the induction of ascites in the animals. It is known from human clinical experience that the growth of abdominal tumors is very painful (fig. 1). Further, ascites fluid accumulation in human patients is associated with abdominal distension, anorexia, nausea, vomiting, respiratory distress, edema, decreased mobility and fatigue. Such individuals are treated for discomfort and pain while never being allowed to progress to advanced stages routinely seen during ascites production in animals.

Animals used for ascites accumulation of MAbs frequently exhibit a spectrum of clinical symptoms including: (1) roughened haircoat, hunched posture, abdominal distention, anorexia, cachexia, anemia; (2) decreased activity and body mass, dehydration, shrunken eyes; (3) difficulty walking, respiratory distress due to an elevated diaphragm; (4) circulatory shock due to excessive fluid removal; (5) decreased venous, arterial and renal blood flow; (6) classical peritonitis; (7) immunosuppression associated with adjuvant use; and (8) up to 20 percent mortality after removal of ascitic fluid. It is not uncommon for fluid to be withdrawn in amounts greater than the entire blood volume of the animal. These symptoms become increasingly severe the longer the animals are allowed to survive (1, 9).

Pathological changes associated with ascites production of MAbs are known for each step in the process. Use of adjuvants produces mild to severe peritonitis and inflammation. Fluid removal may cause hemorrhage, edema, and death. As expected, growth of the ascitic tumors creates a variety of responses including: (1) adhesions of the abdominal wall, bladder, diaphragm, kidneys, liver, seminal vesicles, testicles and ureters; (2) linear lesions in diaphragm muscles; (3) enlarged thoracic lymph nodes and lymphatic obstruction; (4) tumors with extensive hemorrhagic and necrotic areas; (5) disseminated tumors in mesenteric, lumbar, kidney, and testicular regions; (6) centro-lobular liver necrosis; and (7) solid tumors throughout the abdomen. (1, 9)

The significance of the list of abnormalities associated with ascites production is further emphasized by observations that the animals may experience severe pathologic changes in their abdomens and chests, but appear to be clinically normal. By themselves, frequent abdominal injections are known to be a major stressful factor. Further, although abdominal distention is the most obvious consequence of ascites production, severe pain from infiltrated growth of tumors and peritonitis is a significantly more serious problem for the animals.

From all of the above, it is apparent that animals used for ascites production of monoclonal antibodies are routinely subjected to chronic pain and distress. Use of adjuvants further complicates this situation by injuring the animals before the process begins.

In Vitro Alternatives

The most appropriate type of in vitro alternative for each research or diagnostic situation depends largely on the quantity and purity of monoclonal antibody needed. There are, however, some general criteria for rating each system. The ideal method (4, 5):

1. should have expendable material cost similar to that of a mouse.
2. should be a simple technique, requiring no special expertise beyond that for typical cell culture procedures.
3. should not require prior adaptation of the hybridomas or special culture conditions.
4. should have significantly higher concentration and quality of MAbs than for simple stationary cultures.
5. should be a closed, reusable system; free of contamination.
6. should be affordable to all laboratories.
7. should produce adequate quantities of MAbs in a reasonable period of time.

Only a few of the available in vitro methods meet all of these criteria, but there are a wide variety of options available to investigators, ranging from simple, individual cell culture containers to giant, commercial bioreactors.

For individuals needing only small quantities of MAbs, simple, inexpensive stationary cell cultures may be suitable. These involve a variety of flasks and bottles and are simple and easy to use with existing equipment and skills. Batches can be run for days, weeks, or months, depending on the amount of nutrient supplementation and waste removal. These latter processes can be automated. Regardless of the configuration selected, the MAbs must be concentrated after production.

Modifications of the stationary cell cultures primarily involve improved nutrient supply and waste removal, continuous MAb harvesting, and increased hybridoma cell densities. One of the simplest of these is the use of gas permeable tissue culture bags (fig. 2) which provide a practical, economical, and flexible system, using readily available laboratory equipment. In addition to simple convenience, the system is more efficient than rigid tissue culture flasks on a per cell basis and is sig-

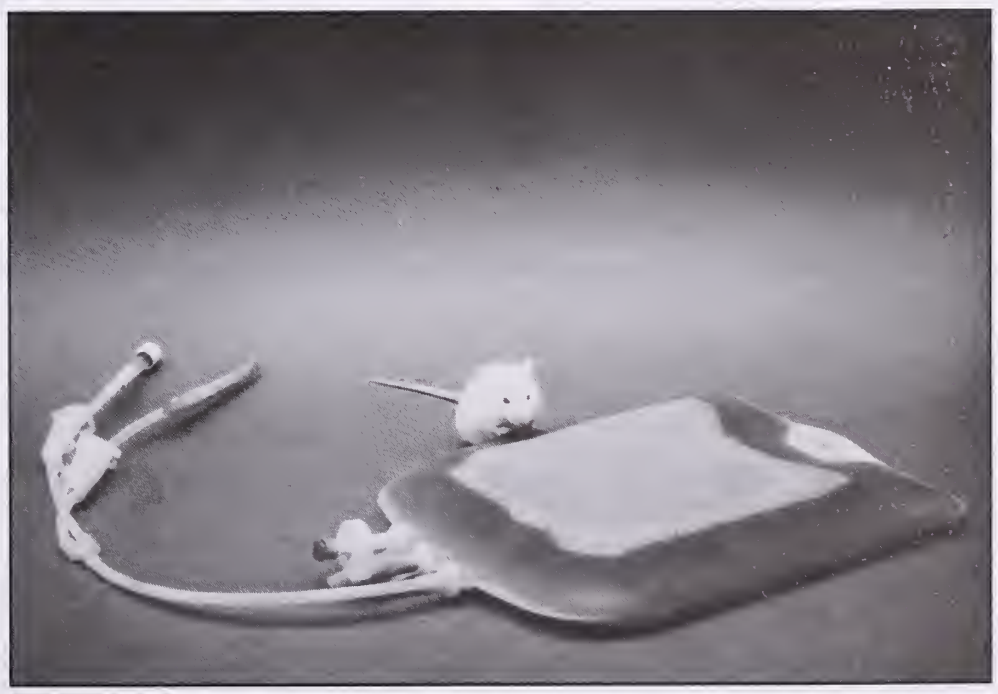

Figure 2. Gas-permeable tissue culture bags are excellent for Mab production.

nificantly less expensive than rodent ascites on a cost per milligram basis and total yield . Because the bags are a closed system with no mechanical parts, they are relatively free of contamination and require little or no monitoring. MAbs are produced in quantities sufficient for small, medium, and large-scale needs (17, 23).

Stationary cultures can also be upgraded by a variety of mechanisms to keep the cells in suspension. These include improvements in stirring and overflow replacement of media (for example, the cytostat (20)) and agitation of cell cultures in roller bottles, spinner flasks, and stirred tanks (2, 14, 19). These alternatives require specific, relatively inexpensive equipment and are simple, fast, and effective.

Roller bottles produce low to medium monoclonal antibody yields at a price similar to that of ascites. Comparative studies of this system that examined 39 different types of hybridomas found that all grew well and produced MAbs (14). Oscillating bubble systems produce higher yields of MAbs, with quantities in each tube exceeding that produced by one ascitic mouse, as well as using equipment that is reusable (19). Due to shear forces in the culture media, spinner flasks may damage the hybridoma cells, and thus are a less effective alternative.

To protect fragile hybridoma cells in suspension cultures and some types of bioreactors, it is possible to encapsulate them in polymers. This provides high yields of MAbs (comparable to ascites methods), with ten-fold increases in cell density over simple suspension systems. Because the cells are isolated from the culture media, it is possible to establish a long-term, continuous operation (up to 40 days in an expanded bed bioreactor). Although available for smaller-scale needs, this technology is primarily used for commercial production of large quantities of MAbs (21).

Dialysis tubing or chambers are used in several different configurations to achieve high hybridoma cell densities with MAb concentrations and purity similar to that found in ascites. The cells may be placed in dialysis bags or other containers within a nutrient chamber to produce up to a gram of MAb within a few weeks. This represents a very inexpensive and simple system which allows several hybridomas to be grown simultaneously (22).

When dialysis tubing is combined with a simple roller-bottle-like mechanism, up to four hybridomas may be grown at once at 10 to 30 fold increase in concentration over stationary cultures. Such compound devices produce MAbs in quantities and purity often equivalent to that of ascites (4).

Combination with a tumbling chamber system provides passive gas exchange with simple, inexpensive, reusable equipment. This approach is capable of producing up to 300 mg of MAb in 21 days and is "universally adaptable in any research laboratory" (10).

One of the most promising in vitro methods for producing high yields of MAbs at a concentration, purity, and cost comparable to mice are the new modular minifermentors (5). These systems meet all the requirements for the perfect in vitro alternative. The hybridoma cells are grown in disposable culture chambers, separated from a reusable media chamber by a gas-permeable dialysis membrane. From 9 to 159 milligrams of MAb can be inexpensively produced in 1 to 4 weeks without the use of serum. Based on current European monoclonal antibody guidelines, these devices have yields equivalent to that of three ascitic mice.

Airlift (packed-bed) bioreactors provide long-term, serum-free production of MAbs in gram amounts. They are particularly cost-effective, since all of the components are reusable (18).

Hollow fiber bioreactors are designed to provide a more physiologically stable environment for the hybridomas and come in both small and large-scale units that produce high cell densities and MAb yields under low or serum-free conditions. Although somewhat more expensive to initially establish than other types of in vitro alternatives, their ability to produce concentrated, relatively pure MAbs at up to half the cost of ascites and in amounts that can be equivalent to 200 mice, 60 large spinner flasks, or a 60 liter fermentor, makes them an attractive option for basic biomedical research and smaller-scale commercial needs. They also require technical expertise to operate without contamination and equipment failures (7, 8).

As noted by the participants in the 1996 ECVAM meeting, there are a

Table 1. Antibody production in different bioreactor systems (Adapted from Stoll et al., 1995 (24)).

System	Maximum Concentration	Productivity	Required for the production of 1 g
In vivo	2200	2	180 mice
In vitro			
T-flask	42	7	400 T-flasks
Stirred-tank bioreactor, batch	47	180[a]	39 days
Stirred-tank bioreactor, fed-batch	120	250[a]	28 days
Hollow fiber reactor	1600	1400	26; 5 days[b]

[a] The total cycle time (innoculation, culture and cleaning) was taken into account.

[b] First number is for first gram; second number is for second gram.

Table 2. Comparisons of different monoclonal antibody production methods. (Adapted from Hendriksen et al., 1996 (6); Kamp amd de Leeuw 1996 (11)).

Production System	Scale	Volume (ml)	Concentration (mg/ml)	Production Time (weeks)[a]	Estimated Cost (per gram)	Quality
Ascites (in vivo)	20-250mg	5-10	< 20	2-3	1000-6000	Low
Stir growth		100-2500	0.01 - 0.1	2 - 3		High
Dialysis membrane	< 50mg	10-25	0.1 - 1.5	2 - 5		High
Roller bottles	< 2g	100-2000	0.01 - 0.2	2 - 6	2000-6000	High
Hollow fiber	0.15-30g	25-1000	0.2 - 30	3 - 12	2700-4000	High
Fermentor	2-100g	< 2000 liters	0.05 - 0.5	2 - 12	1000-6000	High

[a] Excluding immunization, including up-scaling cell cultures.

variety of in vitro approaches to producing MAbs, some of which are briefly described above. For all levels of consumption, replacements for the use of ascites methods are available. Some of the principal characteristics of these alternative options are summarized in tables 1 and 2.

Related Uses of Alternatives

Because in vivo production of monoclonal antibodies involves the largest number of animals and the greatest degree of suffering, it has received the most attention for the development of in vitro replacement alternatives. There are, however, three additional aspects of the process for which humane alternatives are appropriate and needed.

As mentioned above, the initial immunization procedures are most commonly done in vivo, although in vitro techniques are available in many cases. There is a need to further develop these in vitro options so they can be applied to the broad range of MAb needs, not just the production of human-specific antibodies.

Because of serious humane concerns related to its production, the use of fetal calf serum and similar products with in vitro alternatives (either for MAbs or in general) represents another candidate for development of appropriate replacements. Whenever possible, hybridoma cells should be conditioned to grow in serum-free media. This has the additional advantages of reducing the overall expense and post-production processing of the monoclonal antibodies. More than a decade of experience in Europe suggests this type of switch can be easily accomplished with little or no decrease in the production of MAbs. All of the high yield alternatives described above are designed to work effectively without the use of animal serum in their culture media.

Finally, there is a need in the production of hybridomas to replace the use of animal cells with those derived from humans. This is already a critical concern for MAbs produced for therapeutic applications, since murine-derived antibodies are severely limited for effective use in human recipients. For this reason, clinical uses of such antibodies focus on those derived from human cells. To completely replace the use of animals in all steps of MAb production, such concerns need to become more generalized among all producers and consumers of monoclonal antibodies.

The Future Of MAb Production

If current trends continue, the use of animals to produce monoclonal antibodies will eventually be replaced with in vitro alternatives. Although still being refined, new recombinant biotechnology methods based on phage display and combinatorial antibody libraries have the potential to replace the use of animals and animal cells at all stages of the antibody production process, by eliminating the need for the initial immunization and subsequent formation and growth of the hybridoma cells.

These entirely in vitro, high technology approaches mimic and, in some cases, improve upon the natural immune system mechanisms. They accomplished this by creating large libraries of all possible antibodies, including those that would be difficult or impossible to prepare using standard immunization and MAb hybridoma techniques. This is particularly important for the production and use of human MAbs, which are not readily produced using ascites, but are easily derived from the use of recombinant techniques (3, 12, 25, 26).

At present these biotechnology approaches to antibody differentiation and production are not equivalent to or less expensive than ascites or in vitro hybridoma methods. They also require specialized knowledge and skills but will become more competitive and eventually the system of choice as the methodology is refined and developed into commercial kits suitable for use in small to large-scale laboratory applications.

Conclusion

More than 20 years ago, a new in vitro alternative was developed for production of monoclonal antibodies. Because of its simplicity and importance, this technique was widely adopted and used in nearly every field of biomedical research and biotechnol-

ogy. For similar reasons, a new form of laboratory animal cruelty and suffering was also created—the use of ascites methods to produce antibodies.

For the last decade, researchers in Europe and the United States have systematically developed, validated, and adopted multiple in vitro replacements for the use of rodent ascites. This process of technique development has progressed to the point that it is now possible to prohibit use of ascites in all but the most unusual and rare circumstances.

Replacement of animals (either intact, cells, or serum) in production of MAbs will finally allow for implementation of the humane possibilities of Kohler and Milstein's original discovery, replace a method widely acknowledged to be cruel with more humane options, and promote a greater understanding and acceptance of the alternatives approach to planning and conducting biomedical research (16).

For additional information on alternatives in biomedical research, testing and education, or to subscribe to our complimentary newsletter, please contact: John McArdle, Ph.D., Director, Alternatives Research & Development Foundation, 14280 Golf View Drive, Eden Prairie, MN 55346-3000, USA, phone: 612-949-2409, fax: 612-949-2619.

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26. Winter, G., Griffiths, A. D., Hawkins, R. F., and H.R. Hoogenboom (1994). Making antibodies by phage display technology. *Annual Review of Immunology* 12:433-455.Table 1. Antibody production in different bioreactor systems (Adapted from Stoll et al. 1995 (24)) ■

Production of Monoclonal Antibodies Using Mouse Ascites Method

National Institutes of Health
Office for Protection from Research Risks (OPRR) Reports
Number 98-01
Animal Welfare

November 17, 1997

Dear Colleague:

This letter provides guidance to Public Health Service (PHS) awardee institutions and Institutional Animal Care and Use Committees (IACUCs) on avoiding or minimizing discomfort, distress, and pain in the care and use of animals for the production of monoclonal antibodies using mouse ascites antibody production. The Public Health Service Act, the US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, the PHS Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act provide statutory and policy bases for the expectations in these areas (see below for citations).

There is evidence that the mouse ascites method of monoclonal antibody production causes discomfort, distress, or pain. Practical in vitro methods exist which can replace the ascites method in many experimental applications without compromising the aims of the study.

Accordingly, IACUCs are expected to critically evaluate the proposed use of the mouse ascites method. Prior to approval of proposals that include the mouse ascites method, IACUCs must determine that (1) the proposed use is scientifically justified, (2) methods that avoid or minimize discomfort, distress, and pain (including in vitro methods) have been considered, and (3) the latter have been found unsuitable. Fulfillment of this three-part IACUC responsibility, with appropriate documentation, is considered central to an institution's compliance with its Animal Welfare Assurance and the PHS Policy.

The Federal mandate to avoid or minimize discomfort, pain, and distress in experimental animals, consistent with sound scientific practices, is, for all practical purposes, synonymous with a requirement to consider alternative methods that reduce, refine, or replace the use of animals. Consideration of these issues should be incorporated into IACUC review, investigator training, research proposals, and ongoing monitoring of the institutional animal care and use program. IACUCs, acting as agents of institutions, are expected to implement and routinely evaluate these aspects of the institutional animal care and use program to ensure compliance with the PHS Policy.

Because these longstanding requirements are central to the Federal oversight of all animal-related activities in research, testing, and training, this guidance may also be applied more generally to other PHS-supported and non-PHS-supported activities involving animals. Additional references to resources relevant to this issue are enclosed for your information.

Thank you for your attention to these matters. As always, please feel free to contact OPRR at 301-496-7163 if you have questions regarding this correspondence.

Gary B. Ellis, Ph.D.
Director
Office for Protection from Research Risks

Nelson L. Garnett, D.V.M.
Director
Division of Animal Welfare
Office for Protection from Research Risks

Statutory and Policy Bases for Consideration of Alternatives

- **Health Research Extension Act of 1985, Public Law 99-158, at Sec. 495(c):**

"The Director of NIH shall require each applicant for a grant, contract, or cooperative agreement involving research on animals which is administered by the National Institutes of Health ...to include in its application...

"(1)...assurances satisfactory to the Director, NIH that..."(B) scientists, animal technicians, and other personnel involved with animal care, treatment, and use...have available to them instruction or training in the...concept, availability, and use of research or testing methods that limit the use of animals or limit animal distress; and

"(2) a statement of the reasons for the use of animals in research to be conducted with funds provided under such grant or contract."

- **U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training, Principles III, IV, and V:**

"III. The animals selected for a procedure should be of an appropriate species and quality and the minimum number required to obtain valid results. Methods such as mathematical models, computer simulation, and in vitro biological systems should be considered.

IV. Proper use of animals, including the avoidance or minimization of discomfort, distress, and pain when consistent with sound scientific practices, is imperative....

V. Procedures with animals that may cause more than momentary or slight pain or distress should be performed with appropriate sedation, analgesia, or anesthesia..."

- **PHS Policy on Humane Care and Use of Laboratory Animals, IV.A.1.g:**

[The Assurance shall fully describe...] "a synopsis of training or instruction in the humane practice of animal care and use, as well as training or instruction in research or testing methods that minimize the number of animals required to obtain valid results and minimize animal distress, offered to scientists, animal technicians, and other personnel involving animal care, treatment, or use;"

- **PHS Policy on Humane Care and Use of Laboratory Animals, IV.C.1.a.:**

[In order to approve proposed research...the IACUC shall determine that...] "Procedures with animals will avoid or minimize discomfort, distress, and pain to the animals, consistent with sound research design."

- **Guide for the Care and Use of Laboratory Animals, 1996, National Academyof Sciences:**

"Although scientists have also developed nonanimal models for research, teaching, and testing...these models often cannot completely mimic the complex human or animal body, and continued progress in human and animal health and well-being requires the use of living animals. Nevertheless, efforts to develop and use scientifically valid alternatives, adjuncts, and refinements to animal research should continue." (page 1)

"The following topics should be considered in the preparation and review of animal care and use protocols...Availability or appropriateness of the use of less-invasive procedures, other species, isolated organ preparation, cell or tissue culture, or computer simulation..." (page 10)

- **Animal Welfare Act, as amended by the Food Security Act of 1985, Public Law 99-198:**

"[The Secretary shall promulgate...requirements]...for animal care, treatment, and practices in experimental procedures to ensure that animal pain and distress are minimized... that the principal investigator considers alternatives to any procedure likely to produce pain to or distress in an experimental animal..." (Section 13(a)(3)(A)&(B))

"Each research facility shall provide for the training of scientists, animal technicians, and other personnel...training shall include instruction on – (1) the humane practice of animal maintenance and experimentation; (2) research or testing methods that minimize or eliminate the use of animals or limit animal pain or distress;" (Section 13(d))

- **CFR, Title 9, Chapter 1, Subchapter A - Animal Welfare, Sec. 231(d):**

"In order to approve...activities...the IACUC shall determine that...(i) Procedures involving animals will avoid or minimize discomfort, distress, and pain to the animals; (ii) The principal investigator has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources...used to determine that alternatives were not available."

- **CFR, Title 9, Chapter 1, Subchapter A - Animal Welfare, Sec. 232(c):**

"Training and instruction of personnel must include guidance in...(2) The concept, availability, and use of research or testing methods that limit the use of animals or minimize animal distress;"

Resources for Alternatives in Animal Care and Use

- Adjuvants and Antibody Production, ILAR Journal, National Research Council, Institute of Laboratory Animal Resources, Volume 37, Number 3, pp. 92-152, 1995.

- Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing. A bibliography with abstracts prepared by the Toxicology and Environmental Health Information Program, Specialized Information Service, National Library of Medicine, NIH. This document is updated quarterly. To receive the latest copy or be placed on the mailing list, call Vera Hudson at 301-496-1131. Also available on-line at: http://sis.nlm.nih.gov/altanimals.htm

- Altweb is a world wide web site devoted to replacement, reduction and refinement alternatives for research and testing, maintained by the Johns Hopkins Center for Alternatives to Animal Testing (CAAT). The site is: http://www.sph.jhu.edu/~altweb/

- Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies, Information Resource Series No. 3, March 1997, available from the Animal Welfare Information Center (AWIC), NAL, USDA, 10301 Baltimore Avenue, Beltsville, MD 20705. Also available on-line at: http://www.nal.usda.gov/awic/pubs/antibody

- NIH Plan for the Use of Animals In Research, October 1993. Copies available from the Office of Laboratory Animal Research, OER, NIH, 9000 Rockville Pike, Building 1, Room 252, Bethesda, MD 20892. Available on-line at: http://www.sph.jhu.edu/~altweb/science/regs/nihplan.htm

- Office for Protection from Research Risks (OPRR) laboratory animal welfare web page available at: http://www.nih.gov:80/grants/oprr/library_animal.htm

- A Report on Validation and Regulatory Acceptance of Toxicological Test Methods, NIH Publication No. 97-3981, available from the Center for Evaluation of Alternative Toxicological Methods, NIEHS, P.O. Box 12233, Research Triangle Park, North Carolina 27709-12233. Also available on-line at: http://ntp-server.niehs.nih.gov/htdocs/ICCVAM/ICCVAM.html ■

Alternatives in Monoclonal Antibody Production Workshop

by
Christina Goettel-Connolly
Unisyn Technologies
Hopkinton, Massachusetts

On September 24-25, 1997, over 110 participants from research, industry, government, and special interest groups attended the "Alternatives in Monoclonal Antibody Production" workshop, organized by The Johns Hopkins Center for Alternatives to Animal Testing and cosponsored by the National Institutes of Health's Office for Protection From Research Risks. The workshop examined in vitro alternative production techniques, cost, animal welfare, and Institutional Animal Care and Use Committee (IACUC) issues.

The workshop was prompted by the April 23, 1997, American Anti-Vivisection Society (AAVS) petition to the National Institutes of Health (NIH) and the U.S. Department of Agriculture (USDA), requesting that the two institutions follow in the footsteps of their European counterparts to ban in vivo monoclonal antibody (MAb) production. According to the AAVS, up to 1 million animals per year are sacrificed in the United States for antibody production. [Ed. note: According to a 1991 report by the Business Communications Company, an estimated 2.6 million mice were used worldwide for the production of MAbs. The United States share was estimated at 40 percent of the world total.]

Representatives from USDA and NIH were present at the workshop to make a formal response to the petition. Louis Sibal, D.V.M., Director of the NIH Office of Laboratory Animal Research concluded that although alternatives are "scientifically acceptable, reasonable, and practically available," NIH will not approve the ban. According to Jerry DePoyster, D.V.M., Veterinary Medical Officer, USDA, Animal Care, USDA expects its field personnel to make sure an alternatives search has been done if the researcher plans to work with a regulated animal covered by the Animal Welfare Act (AWA). However, USDA animal welfare regulations currently excludes rats and mice from coverage and USDA does not expect to be changing its list of regulated animals in the near future.

Several conclusions and recommendations were made at the workshop. First, in vitro alternatives for MAb production should be the accepted method, with the ascites method being the exception. If the ascites method must be used, the investigator should justify this carefully and take care to minimize stress and pain. Consequently, the role of the IACUC is becoming pivotal in advising and educating investigators about proper alternatives.

Another conclusion was that the procedure is painful and causes suffering. According to data presented, mice used for ascites production experience problems such as respiratory distress, circulatory shock, difficulty walking, anorexia, dehydration, and peritonitis. A topic of debate was (and still is) the permitted number of abdominal taps, since fluid removal may cause hemorrhage, edema, and death. Furthermore, many mice develop solid tumors during the process, rendering them useless for production.

The workshop also determined that alternatives not only produce adequate amounts of MAbs, but are also cost– and time–efficient. Leading researchers presented cost and production data from alternative in vitro methods such as hollow fiber bioreactors, the miniPERM bioreactor, and gas–permeable tissue culture bags that confirmed the feasibility of these methods.

Unisyn Technologies (Hopkinton, MA) displayed the new Cell-Pharm System 100 hollow fiber bioreactor system and the miniPERM bioreactor. Leading researchers presented data which confirmed that on a cost per milligram basis, the miniPERM bioreactor is competitive or less expensive than ascites when factoring in mouse per diem costs and other associated costs such as investigator /IACUC time needed to write/review each ascites proposal. These data also indicated in vitro antibody concentrations equivalent to ascites, without the contamination of adventitious murine viruses or nonspecific proteins.

Another topic discussed was the increasingly important trend towards central hybridoma core facilities. More and more core facilities and contract laboratories are expanding their current services to include in vitro MAb production. Some offer in vitro production methods only. These core facilities offer in vitro services to researchers with personnel, cost, and/or space constraints.

The workshop not only confirmed the feasibility and easy availability of alternative methods, but also served as an excellent information resource for anyone interested in the 3Rs: reduction, refinement, and replacement. The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) hosts AltWeb, a website dedicated entirely to alternatives. The website contains links to many other related sites and can be reached at the address http:/infonet.welch.jhu.edu/~caat Another helpful resource is the August 1997 *Information Resources for Adjuvants and Antibody Production: Comparisons and Alternative Technologies* developed by USDA's Animal Welfare Information Center. This publication can be found at http://www.nal.usda.gov/awic/pubs/antibody

For additional information on Unisyn products, please contact Christina Goettel-Connolly, Unisyn Technologies, Inc., 25 South Street, Hopkinton, MA 01748-2217 USA, phone: (508) 435-2000, fax: (508) 435-8111, World Wide Web: http://www.unisyntech.com

[Ed. note: For additional information about other products or companies providing equipment or supplies for in vitro production of MAbs, please visit http://www.nal.usda.gov/awic/pubs/antibody/company.htm on the AWIC web site.]

Enrichment in Group-Housed Laboratory Golden Hamsters

by
Cheryl Arnold, Ph.D. and
Robert D. Westbrook, Ph.D.
Hendrix College, Conway, Arkansas

Summary

We provided group-housed golden hamsters with enrichment items (either a mason jar or pvc pipe) for 2 weeks at a time in an attempt to reduce aggression. Compared to controls, who had nothing extra in their cages, enriched hamsters showed more varied behavior and less aggression toward their cagemates. The hamsters preferred jars to pipes. Based on our observations, we recommend that when enrichment items are given to group-housed hamsters, multiple items be included in each cage and groups be monitored, as their aggression levels change with age.

Introduction

Laboratory golden hamsters (*Mesocricetus auratus)*are often group-housed. While this may seem odd for what is considered a solitary species (1), many golden hamsters do quite well under these conditions. We've even found that in spite of the common aggression and wounding, both male and female golden hamsters prefer social contact to solitary conditions (2,3). Given this state of affairs, we believe that laboratories which group-house their golden hamsters should find ways to reduce the aggression among cagemates. We tested two readily available, durable, and inexpensive items to see if they reduced aggression.

Subjects and Materials

We received six pregnant females (Sprague-Dawley, Indianapolis, Indiana) which all gave birth 2 days after arrival. We housed mothers with their litters in polypropylene cages (18.5 inches x 10 inches x 8 inches high) with cedar shavings, brown paper toweling (for nests), and free food and water.

At day 21 postpartum, we weaned pups into 12 permanent groups of four (same-sexed) littermates and housed them in the same type of cages with cedar shavings and free food and water. Lights were on a 12 hours on–12 hours off cycle, and cages were changed twice a week. Each litter contributed one cage of females and one cage of males to the study, and no animals died.

For enrichment, we used mason jars and pvc pipes (fig. 1). We purchased the pint-sized, clear glass jars at a grocery store and the 2-inch diameter, white plastic pipes ("T" joints) at a hardware store.

Figure 1. PVC pipe and glass jars were evaluated as enrichment items.

Procedure

We rotated each cage through a 2-week treatment of the jar (J), pipe (P), and control (C) conditions using all possible treatment orders (JPC, JCP, PCJ, PJC, CJP, CPJ). This controlled for possible confounding between age and treatment. For example, if all hamsters had experienced the J condition last, and if aggression had been lowest in the J condition, we would not have known if this resulted from the jar or from older hamsters being less aggressive. In this part of the study, only one enrichment item was in each cage at any time.

We collected data on the last 2 days of each treatment to minimize novelty effects. Using one-zero scoring, we recorded whether or not hamsters showed object interaction or aggression during 60 consecutive 10-second intervals (10 minutes). Object interaction was defined as any hamster physically interacting with an enrichment item. We excluded non-contact, external sniffing of the item, and apparent incidental contact, such as leaning against the item while sleeping. Aggression was defined as two or more hamsters tumbling rapidly or "balling up" and biting one another. The experimenter observed each cage in a varied order for 10 minutes during the morning (0700-

0900), afternoon (1300-1500), and evening (1900-2100) hours of both days. This provided a total of 60 minutes of data per cage per condition. During the dark periods, we used two 25-watt red lights for conducting observations.

For statistical purposes, we treated the hamsters in each cage as one subject (N = 12) and we used the number of intervals in which a behavior was scored as a measure of frequency. It is worth noting that these frequencies are underestimated because we only recorded whether or not a behavior occurred (a score of one or zero) in each interval, and not how many times each behavior occurred.

Results and Discussion

The hamsters used both enrichment items, but preferred the jars. They interacted with the jars an average of 184 intervals, more than twice as often as they interacted with the pipes (85 intervals). As figure 2 clearly shows, object interaction decreased with age. Although we did not collect data on additional behaviors, our impression was that hamsters in the control conditions spent more time sleeping and eating. Enrichment appeared to increase species-typical behaviors like scent marking, gnawing, hoarding, and digging.

Hamsters used the items in different ways than we envisioned. Most commonly, they stood atop the jar or pipe while peering out from, or gnawing on, the stainless steel bar lids. Several groups used the jars for urinating or hoarding their food. These two tendencies made cage cleaning a lot easier. Young hamsters regularly slept together in the jar, but this behavior decreased as the animals grew. Jars were also scent marked, gnawed on, hid in, and defended. Pipes did not elicit nearly as many behaviors. Some hamsters spent a lot of time tunneling through them, but this was rare. Like jars, pipes were scent marked and gnawed on, but only occasionally hid in and rarely defended. Hamsters may have preferred jars because the jars greater height, as compared to pipes, made it easy to look outside the cage. Other reasons they may have favored the jars were the security of a single entrance, their transparency, or their versatility.

The effects on aggression were often dramatic, although groups varied greatly in their aggression levels. Because of this, we've reported median data in this section rather than averages. The median is the halfway point, and it gives a truer estimate of the central data point than the average, or mean, when extreme values are present and the data don't fit a normal curve. Overall, aggression decreased with age (7 of the 12 cages showed no aggression during data collection at 9 weeks) and with enrichment. Aggression dropped from a median of 24.5 intervals at 5 weeks to 3.5 at 7 weeks to 0 at 9 weeks (fig. 3). Median values were 9 intervals in the control condition, 3.5 in the pipe condition, and 2 in the jar condition (fig. 4). Enrichment items decreased aggression the most in young hamsters. Five-week old hamsters showed a median of 47 intervals of aggression in the control condition, but only 16 in the jar condition and 11 in the pipe condition.

Our control and jar conditions showed the pattern of highest aggression at 5 weeks, lowest aggression at 7 weeks, and slightly increased aggression at 9 weeks (see fig. 5). Our pipe condition did not. Seven-week old hamsters with pipes spent a median of 24 intervals fighting (up from 11), but 9-week old hamsters with pipes spent a median of 0 intervals fighting. One explanation for this puzzling result was our small sample size – the four most highly aggressive groups just happened to be assigned to pipes at 7 weeks of age. Another possible factor was that by this time our females were cycling, and we may have collected data on these females on their more aggressive days. We did not attempt to verify day of cycle. Still, this unexpected aggression points to the importance of monitoring animals who are given enrichment. In our case, one group of females increased their aggression from two intervals with the jar (at 5 weeks) to 29 intervals with the pipe (at 7 weeks).

Figure 2. Mean number of intervals engaged in object interaction, by enrichment condition and age.

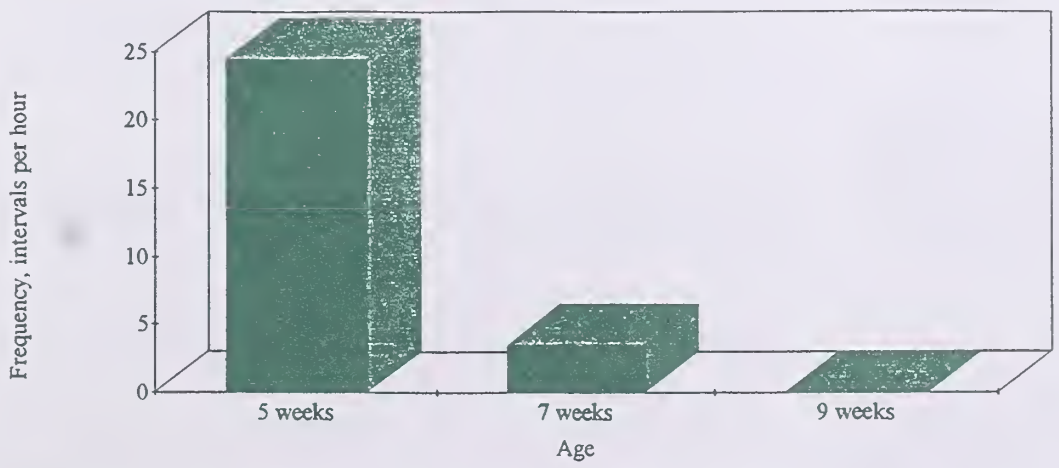

Figure 3. Median number of intervals engaged in aggression, by age.

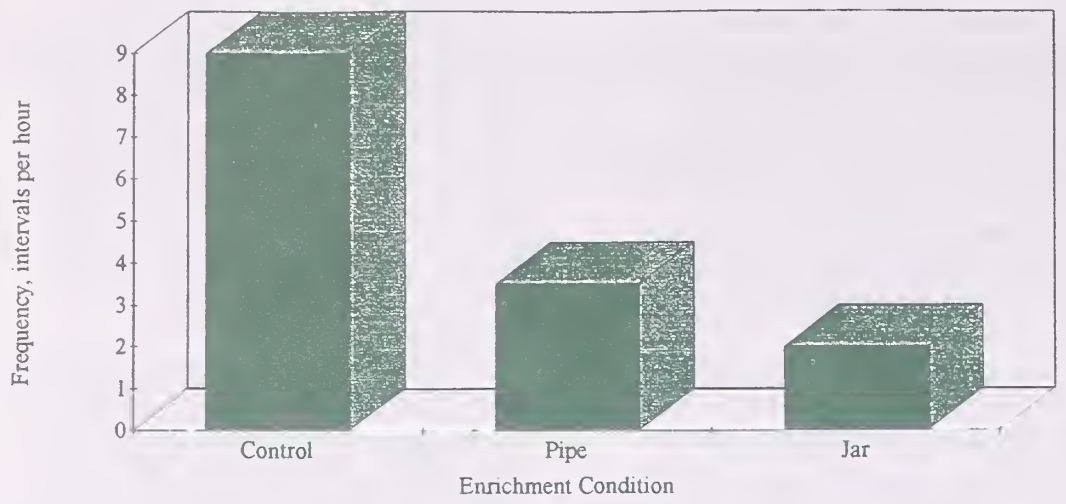

Figure 4. Median number of intervals engaged in aggression, by enrichment condition.

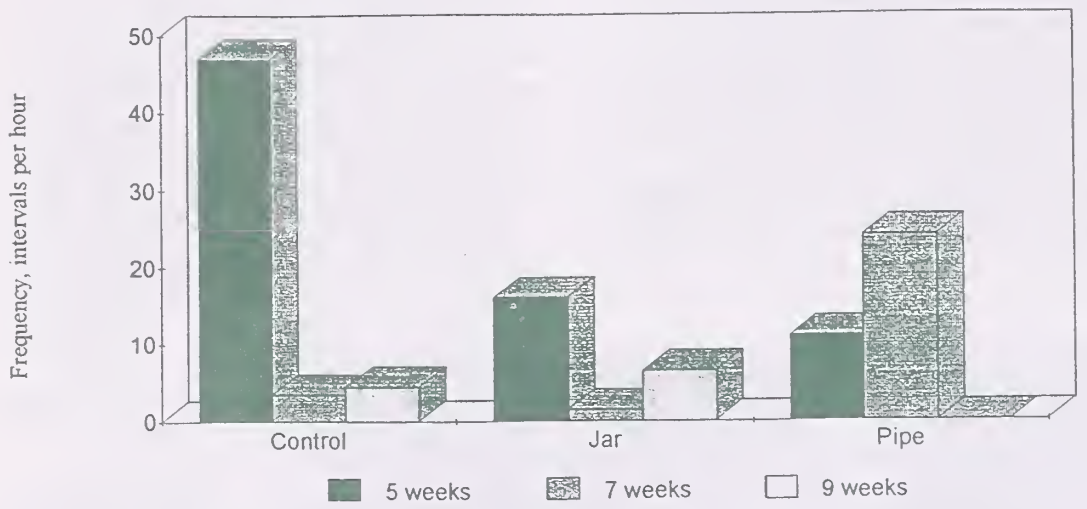

Figure 5. Median number of intervals engages in aggression, by enrichment condition and age.

We ran a followup study to see if the preference for jars lasted. To do this, we removed enrichment items from all hamsters for 3 additional weeks and then added both a jar and a pipe to each cage for 2 weeks. Data were collected as before and showed that hamsters still preferred the jars (an average of 64 intervals spent in jars, and 43 spent in pipes) and aggression decreased further (median 0 intervals). It's also interesting that, whereas we found males to be the more aggressive sex in the first study (total aggressive intervals: males 336, females 152), we found the reverse in the follow-up study (total aggressive intervals: males 0, females 40). Hormonal changes are likely responsible for the fact that males and females often show different rates of aggression at different times in their lives. Although there are several differences between the living conditions we tested and the conditions that most hamster aggression studies use, our results support the notion that adult females are generally more aggressive than adult males in this species (1,4).

Conclusion

Providing group-housed golden hamsters with simple enrichment items can substantially reduce cagemate aggression, especially when animals are weanlings. Because our enriched animals expressed more species-typical behaviors, enrichment appears beneficial for singly-housed hamsters as well. However, it seems important to determine whether singly-housed hamsters act more aggressively toward handlers when easily defendable items, like jars, are present, before a facility mandates their use. (For a discussion of hamster-human interaction, see reference 3).

In the current study, group-housed hamsters competed for a single enrichment item. Some colleagues have suggested that four items per cage (specifically, one jar per animal) would have been ideal. We disagree. Besides the space and safety considerations that multiple, movable jars would bring, we believe it is unlikely that animals would forego fighting and automatically establish individually "owned" jars. Arnold and Estep (2) observed that when five male hamsters shared a five-cage chamber provisioned with separate food and water supplies, most animals hoarded their food into a communal pile, slept together, and urinated in one or two locations. They did not act territorial. However, we agree that multiple items should reduce competition and might further reduce cagemate aggression. We recommend that caretakers closely monitor cages with enrichment items as the hamsters age and add items, or remove problem animals, when necessary.

Acknowledgments

We'd like to thank students Kevin Hoggard for helping with the animals and Aaron Kemp for proposing the final preference test.

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Legislation cont'd from p.1

such prior comment is not practicable, in the period immediately following the appointments. The Academy shall make its best efforts to ensure that (A) no individual appointed to serve on the committee has a conflict of interest that is relevant to the functions to be performed, unless such conflict is promptly and publicly disclosed and the Academy determines that the conflict is unavoidable, (B) the committee membership is fairly balanced as determined by the Academy to be appropriate for the functions to be performed, and (C) the final report of the Academy will be the result of the Academy's independent judgment. '(2) The Academy shall determine and provide public notice of committee meetings that will be open to the public. '(3) The Academy shall ensure that meetings of the committee to gather data from individuals who are not officials, agents, or employees of the Academy are open to the public, unless the Academy determines that a meeting would disclose matters described in section 552(b) of title 5, United States Code. The Academy shall make available to the public, at reasonable charge if appropriate, written materials presented to the committee by individuals who are not officials, agents, or employees of the Academy, unless the Academy determines that making material available would disclose matters described in that section. '(4) The Academy shall make available to the public as soon as practicable, at reasonable charge if appropriate, a brief summary of any committee meeting that is not a data gathering meeting, unless the Academy determines that the summary would disclose matters described in section 552(b) of title 5, United States Code. The summary shall identify the committee members present, the topics discussed, materials made available to the committee, and such other matters that the Academy determines should be included. '(5) The Academy shall make available to the public its final report, at reasonable charge if appropriate, unless the Academy determines that the report would disclose matters described in section 552(b) of title 5, United States Code. If the Academy determines that the report would disclose matters described in that section, the Academy shall make public an abbreviated version of the report that does not disclose those matters. '(6) After publication of the final report, the Academy shall make publicly available the names of the principal reviewers who reviewed the report in draft form and who are not officials, agents, or employees of the Academy.

- **H.R. 2807 To amend the Rhinoceros and Tiger Conservation Act of 1994 to prohibit the sale, importation, and exportation of products labeled as containing substances derived from rhinoceros or tiger.**

Introduced November 4, 1997, by Jim Saxton (R-New Jersey) and referred to the Committee on Resources. This act may be cited as the "Rhino and Tiger Product Labeling Act."

Congress finds the following: (1) The populations of several magnificent and unique endangered species of rhinoceros and tigers, such as the Indian rhinoceros, the Javan rhinoceros, the African black rhinoceros, and all of the tiger subspecies, continue to decline. (2) Growing demand throughout the world for wildlife and wildlife parts and products has created a market in which commercial exploitation has threatened certain rhinoceros and tiger populations. (3) There are insufficient legal mechanisms enabling the United States Fish and Wildlife Service to forcefully interdict products that are labeled as containing substances derived from rhinoceros or tiger species and prosecute the merchandisers for sale or display of those products. (4) Although approximately 77,000 import and export shipments occur annually in the United States, the United States Fish and Wildlife Service is able to maintain only 92 wildlife inspectors at 30 ports of entry, including 13 designated ports, to monitor the shipments. (5) Wildlife inspectors are able to physically inspect only an estimated 5 to 10 percent of all import and export shipments, making the rate of detection of contraband wildlife products extremely low.

Section 7 outlines prohibitions relating to labeling. "No person shall sell, import, or export any product labeled as containing any substance derived from any species of rhinoceros or tiger. Any person who knowingly violates this prohibition shall be fined under title 18, United States Code, imprisoned for not more than 1 year, or both."

- **H.R. 752 To amend the Endangered Species Act of 1973 to ensure that persons that suffer or are threatened with injury resulting from a violation of the Act or a failure of the Secretary to act in accordance with the Act have standing to commence a civil suit on their own behalf.**

Introduced February 13, 1997, by Helen Chenoweth (R-Idaho) and referred to the Committee on Resources. This act may be cited as the "Citizen's Fair Hearing Act of 1996."

Congress finds the following: (1) The Endangered Species Act of 1973 grants broad regulatory authority to various agencies to take actions to protect, preserve, and recover species of plants and animals determined to be in danger of extinction or threatened with becoming so within the foreseeable future. (2) Recently, private property owners and other persons that have been adversely impacted by Federal agency actions under the Endangered Species Act of 1973 have sought to bring civil actions for judicial review of those agency actions. The United States Circuit Court of Appeals for the 9th Circuit has found that plaintiffs in those actions do not have standing to bring the suits, because they do not fall into the zone of interests protected by the Endangered Species Act of 1973.

Section 3. Giving persons with affected economic interests equal standing to sue under the Endangered Species Act of 1973. "Any person that satisfies the requirements of the Constitution and demonstrates having suffered or being threatened with economic or other injury resulting from a violation of the Act or a failure of the Secretary to act in accordance with the Act is deemed to be within the zone of protected interests of this Act and shall have standing to commence a civil suit on his or her own behalf."

- **H.R. 478 To amend the Endangered Species Act of 1973 to improve the ability of individuals and local, State, and Federal agencies to comply with that Act in building, operating, maintaining, or repairing flood control projects, facilities, or structures.**

Introduced on January 21, 1997, by Wally Herger (R-California) and referred to the Committee on Resources. This act may be cited as the "Flood Prevention and Family Protection Act of 1997."

The purpose of this act is to improve the ability of individuals and local, State, and Federal agencies to comply with the Endangered Species Act of 1973 in building, operating, maintaining, or repairing flood control projects, facilities, or structures to address imminent threats to public health or safety or catastrophic natural events or to comply with Federal, State, or local public health or safety requirements.

Section 3 lists exemptions from consultation and conferencing requirements of Section 7(a) of the Endangered Species Act of 1973 (16 U.S.C. 1536(a)). An activity of a Federal or non-Federal person is not a taking of a species if the activity consists of building, operating, maintaining, or repairing a Federal or non-Federal flood control project, facility, or structure to address a critical, imminent threat to public health or safety; or to address a catastrophic natural event; or to comply with Federal, State, or local public health or safety requirements; or consists of routine operation, maintenance, rehabilitation, repair, or replacement of a Federal or non-Federal flood control project, facility, or structure, including operation of a project or a facility in accordance with a previously issued Federal license, permit, or other authorization.

- **S. 901 To provide Federal tax incentives to owners of environmentally sensitive lands to enter into conservation easements for the protection of habitat; to amend the Internal Revenue Code of 1986 to allow a deduction from the gross estate of a decedent in an amount equal to the value of real property subject to an endangered species conservation agreement; and for other purposes.**

Introduced June 12, 1997, by Dirk Kempthorne (R-Idaho) and referred to the Committee on Finance. This act may be cited as the "Endangered Species Habitat Protection Act of 1997."

The Senate finds and declares the following: The majority of American property owners recognize the importance of protecting the environment, including the habitat upon which endangered and threatened species depend. (2) Current Federal tax laws discourage placement of privately held lands into endangered and threatened species conservation agreements. (3) The Federal Government should assist landowners in the goal of conserving endangered and threatened species and their habitat. (4) If the environment is to be protected and preserved, existing Federal tax laws must be modified or changed to provide tax incentives to landowners to attain the goal of conservation of endangered and threatened species and the habitats they depend upon.

Section 3 allows for enhanced tax deductions for the denotation of a conservation easement. Section 4 includes exclusions from estate for real property subject to endangered species conservation agreement. Sections five outlines income tax incentives to preserve land to protect endangered species.

- **S. 1033 A bill making appropriations for Agriculture, Rural Development, Food and Drug Administration, and Related Agencies programs for the fiscal year ending September 30, 1998, and for other purposes.**

Introduced July 17, 1997, Thad Cochran (R-Mississippi), and referred to the Committee on Appropriations. Senate Report No. 105-51 issued.

"For necessary expenses to enable the Agricultural Research Service to perform agricultural research and demonstration relating to production, utilization, marketing, and distribution (not otherwise provided for); home economics or nutrition and consumer use including the acquisition, preservation, and dissemination of agricultural information; and for acquisition of lands by donation, exchange, or purchase at a nominal cost... "

Appropriation language included for buildings and facilities, Cooperative State Research, Education, and Extension Service, Animal and Plant Health Inspection Service and other programs. ■

USDA Facilitates the Formation of Marine Mammal Expert Panel

RIVERDALE, MD, Nov. 24, 1997 - The U.S. Department of Agriculture with the support of the Free Willy Keiko Foundation (FWKF) has successfully facilitated the formation of an expert panel to independently review the health of Keiko, a killer whale belonging to the FWKF in Newport, Oregon.

"While an Oct. 8 Animal and Plant Health Inspection Service inspection of FWKF facilities turned up no Animal Welfare Act violations, we believe an independent health evaluation will be in the best interests of the whale and leave no room for doubt as to Keiko's current health," said W. Ron DeHaven, acting deputy administrator for animal care with APHIS, a part of USDA's marketing and regulatory programs mission area.

The panel members include:

Dr. Joseph Geraci of the National Aquarium in Baltimore;
Dr. Jim McBain of Sea World;
Dr. Al Smith of Oregon State University;
Dr. Jeff Stott of the University of California;
Dr. Barbara Kohn of APHIS' Animal Care program; and
Mr. Bud Krames of Dolphin Quest.

The purpose of the panel is to assess and evaluate Keiko's current health by gathering veterinary and behavioral information. The panel will not evaluate Keiko's status as a possible candidate for release into the wild.

The panel expects to complete its evaluation by Dec. 15 and release the results soon after.

Note to Editors: The panel members will not discuss the study nor the results until after the results are released. All media queries should be directed to Jim Rogers, APHIS LPA, (301) 734-8563.

USDA news releases, program announcements, and media advisories are available on the Internet. Access the APHIS Home Page by pointing your Web browser to http://www.aphis.usda.gov and clicking on "APHIS Press Releases." Also, anyone with an e-mail address can sign up to receive APHIS press releases automatically. Send an e-mail message to majordomo@info.aphis.usda.gov and leave the subject blank. In the message, type subscribe press_releases ■

Announcements...

SERVICES

- **Database Searching Service Now Available in Australia**

Scientific Information Service (SIS) has been established by Wendy van Dok, Ph.D., a Melbourne toxicologist, to provide a scientific literature searching service that can provide information on alternatives to the use of animals or on ways to minimize pain and distress during animal experiments. Using databases available through several commercial organizations, SIS can provide continuous, comprehensive coverage of the international scientific literature. Results can be e-mailed to ensure quick delivery, and translation services are available. SIS can also compile bibliographies on any topic. For more information, contact Wendy van Dok, GPO Box 2579W, Melbourne, Vic. 3001 Australia, phone: (03) 9802 7211, fax: (03) 9802 0772, e-mail: wvandok@netspace.net.au

PUBLICATIONS

- **Animal Alternatives, Welfare, and Ethics: Proceedings of the 2nd World Congress on Alternatives and Animal Use in the Life Sciences**

The 2nd World Congress, held in Utrecht, The Netherlands, October 20-24, 1996, provided a global overview of the current status of reduction, refinement, and replacement alternatives. This publication, edited by L.F.M. van Zutphen, Utrecht University, and M. Balls, European Center for the Validation of Alternative Methods, provides an up-to-date overview of the various aspects dealing with the development, validation, and use of animal alternatives. In addition, current topics on animal welfare and ethical aspects of animal experiments are covered. Topics include humane endpoints, alternative animal models, noninvasive methods, databases, alternatives in toxicology, monoclonal and polyclonal antibodies, validation of alternative methods, regulatory viewpoints, etc. The publication has extensive subject and author indexes. The cost is NLG (Dutch guilders) 545 or US$ 340.75. 1260 pages. ISBN 0-444-82424-3

It is available from Elsevier Science, P.O. Box 945, New York, NY 10159-0945, phone: (212) 633-3730 or toll-free (888) 437-4636 (North America only), fax: (212) 633-3680,

e-mail: usinfo-f@elsevier.com Elsevier Science, P.O. Box 211, 1000 AE Amsterdam, The Netherlands, phone: +(31) 20-485-3757, fax: +(31) 20-485-3432, e-mail: nlinfo-f@elsevier.nl

- **Animals in Philosophy and Science**

This series of texts will include topics such as animal consciousness, evolutionary psychology, animals in society, and cultural images of animal ethics. The first volume, *Animal Consciousness and Animal Ethics,* contains 15 chapters arranged in 3 sections—Philosophy and Animal Consciousness, Science and Animal Consciousness, and Ethics and Animal Consciousness. The first part of the book covers the concept of consciousness in general and the way to acquire knowledge of conscious animal experiences. Part two covers the area of ethology and neuroscience with a discussion of theoretical and experimental approaches to animal consciousness, animal experiences, and their implications for animal welfare. Part three covers whether and how our treatment of animals should be based on animal experiences or whether other concepts are needed to give direction to humane treatment of animals. 264 pages.

To order or for additional information contact Van Gorcum Publishers, P.O. Box 43, NL-9400 AA Assen, The Netherlands, phone: 31 592 379555 fax: 31 592 372064, e-mail: assen@vgorcum.nl In the United States and Canada: contact Books International, Inc., P.O. Box 605, Herndon, VA 22070, phone: (703) 661-1500, fax: (703) 661-1501. The cost is NLG 59.90 or US$34.

- **The National Survey of Laboratory Animal Use, Facilities, and Resources**

This survey was conducted in 1995 (see *AWIC Newsletter* Vol.5 #4 Winter 1994/95) to provide the National Institutes of Health (NIH) with information to "use in the planning and institution of programs to improve the quality and availability of laboratory animals, promote institutional care and humane treatment , improve facilities, and establish training programs in laboratory animal medicine. It will also contribute to the understanding of the effects of Federal, state, and local laws, regulations, and policies enacted to ensure humane care and to regulate the supply and cost of laboratory animals." The data collected is from fiscal year 1993. To order, contact the Office of Science and Health Reports, NIH, fax: (301) 480-3558, e-mail: ospio@ep.ncrr.nih.gov

- **Directory of Comparative Medicine Resources**

Comparative Medicine Resources, a new 94-page directory, describes more than 60 National Center for Research Resources (NCRR) supported resources and training awards available to the scientific community. These resources include seven Regional Primate Research Centers, special animal colonies and models, and animal information services. Available animal models include genetically defined strains of *Drosophila melanogaster* and *Caenorhabditis elegans*, laboratory reared *Aplysia californica*, transgenic mice and rats, specific-pathogen-free monkeys, and chimpanzees.

The directory summarizes each resource's current research efforts; provides details about available animal models, specimens, or services; lists key staff members; and describes procedures for accessing certain resources. To obtain a free copy of *Comparative Medicine Resources* directory, contact the Research Resources Information Center, 1601 Research Blvd., Rockville, MD 20850 USA, phone: (301) 838-6261, fax: (301) 838-6252, e-mail: rric@vitro.com The latest updates of the directory can be viewed on the NCRR web site at http://www.ncrr.nih.gov/ncrrprog/cmpdir/content.htm

WORKSHOPS, CLASSES, SYMPOSIUMS, ETC.

- **NIH National Animal Welfare Workshop**

On January 16, 1998, the Office for Protection from Research Risks and the Scientists Center for Animal Welfare (SCAW) will co-sponsor a 1-day workshop on *Nonaffiliated and Nonscientist Members of IACUCs.* The workshop will be held at the NIH Natcher Conference Center in Bethesda, Maryland. For more information or to register, contact Lee Krulisch, SCAW, 7833 Walker Drive, Suite 340, Greenbelt, MD 20770, phone: (301) 345-3500, fax: (301) 345-3503, e-mail: scaw@erols.com

- **International Course on Laboratory Animal Science—Utrecht University**

A 2-week intensive course on laboratory animal science will be organized at the Department of Laboratory Animal Science, Utrecht University, The Netherlands, from June 8 to 19, 1998. The objective of this course is to present basic facts and principles that are essential for the humane use of animals and for the quality of research. The contents of this course are in line with recommendations of the Federation of European Laboratory Animal Science Associations (FELASA) regarding the training of young scientists whose research involves the use of vertebrate animals. The course may also be of interest for those who intend to set up a similar course at their location. For this purpose, during the course,

the acquisition of teaching materials can be discussed with the course committee. For information or for application forms, please contact Prof. dr. L. F. M. van Zutphen or Mrs. Marianne Albers, Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.166, 3508 TD Utrecht, The Netherlands, phone: 31-30-2532033, fax: 31-30-2537997, e-mail: pdk@pobox.ruu.nl

• INVITOX - 10th International Workshop on In Vitro Toxicology

INVITOX is a workshop on in vitro toxicology held in Europe every 2 years. The aim of the workshop is to promote the exchange of views, information, and experience among scientists involved in the development and use of in vitro methods in toxicology and toxicity testing. The program will consist of six main sessions focused on recent advances, production and use of transgenic cells, acquisition and use of human cells, toxicity of complex materials, in vitro models for investigation of chronic toxicity and reversibility, and other topics. However, abstracts may be submitted on any aspect of in vitro toxicology. The meeting will be held at Sparsholt College, Hampshire, United Kingdom, from September 14-18, 1998. The number of participants is restricted to 170. For additional information, contact Caroline Sumner, INVITOX 98 Secretariat, Meetings Management, The Chestnuts, 1st Floor, 18 East Street, Farnham, Surrey GU9 7SD, United Kingdom, phone: +44 (0) 1252 726066, fax: +44 (0) 1252 723303, e-mail: jherriot@meetingsmgmy.u-net.com, World Wide Web: http://www.elsevier.nl/locate/invitox98

• 1998 CALAS/ACLAS Symposium and Workshops

The 38th annual Symposium of the Canadian Association for Laboratory Animal Science will be held June 21-24, 1998, at the Coast Plaza Hotel in Calgary, Alberta. The theme is "We Care: Training for Excellence. There will be forums on "Working with Industry" and "Training and the Three R's". Schedules workshops include: Laparoscopic Surgery; Experimental Design; Health, Safety and Liability in a Research Facility; Cardiovascular and Orthopedic Procedures; Immunological Diagnostic Procedures; Identification of Animals; Handling/Restraint Procedures in Cattle, Sheep and Goats; and Maintenance and Repair of Cage Wash and Surgical Equipment.

For more information contact Dr. Don McKay, CALAS/ASCAL, Biosciences Animal Service, CW 401 Biological Sciences Biulding, Edmonton, Alberta, Canada, T6G 2E9, phone: (403)492-5193, fax: (403)492-7257, e-mail: dmckay@gpu.srv.ualberta.ca

RESOURCES

• CD-CANIS from Vetstream

CD-CANIS is a user-friendly, multimedia veterinary encyclopedia that was designed by clinical veterinarians. Disciplines covered by this CD-ROM include anesthesia, clinical pathology, behavior, medicine, parasitology, surgery, anatomy, diagnostic imaging, husbandry, microbiology, preventative medicine, pharmacology and therapeutics, etc. All major body systems are featured. All materials have been peer reviewed before their inclusion. The information retrieved is in full text with more than 2,000 pictures, video clips, sounds, and graphics. The CD is easy to search using several different search mechanisms. Each subscriber can receive a personalized copy of CD-CANIS to enable specific updates of topics of interest to be highlighted. Every 2 months, subscribers receive a new CD with fully revised and updated information. For more information, contact Mark Johnston, Vetstream plc, Langford Arch, Sawston, Cambridge, CB2 4EG United Kingdom, phone: + 44 (0) 1223 500123, fax: +44 (0) 1223 506565, e-mail: enquiries@vetstream.co.uk

• Instructional Software Catalog

The University of California Davis School of Veterinary Medicine offers a series of programs on CD-ROM for veterinary medical education. These programs are just a sampling of the more than 180 instructional programs developed by the Computer Assisted Learning Facility (CALF) for use in the school's veterinary curriculum. Programs include *The Equine Foot, Veterinary Neurosciences, Poisonous Plants, Veterinary Neuropathology, ParasitoLog, Equine Osteology Atlas and Quiz, Virtual Heart, Veterinary CPR Simulator,* and *Canine Osteology Atlas and Quiz.* For more information, contact CALF at (916) 752-2477 or e-mail: rhayes@ucdavis.edu or jbkasper@ucdavis.edu

• Sheffield BioScience Programs

Simulations of undergraduate laboratory experiments in physiology and pharmacology.

These generate simulated tissue responses, either from actual experimental results or from predictive models. Data are presented on the monitor screen, in high resolution graphics, in a form comparable to that in the real experiment such as storage oscilloscope or scrolling chart recorder. Students are expected to simulate many of the tasks associated with practical class teaching such as determining experimental parameters (from easy-to-use, Windows-like menus), and collecting data in much the same way as they would in the laboratory. They work at their own pace, and most students take readily to this form of teaching, often requesting to use the software in their own time for independent learning. The simulation programs make full use of color graphics and will run under a range of graphics drivers (CGA, EGA, VGA etc) on any IBM compatible machine. They are sold complete with multiuser license and documentation (program manual and, where appropriate, student's workbook and tutor's notes). All text in the programs may be edited by the teacher. This requires access to a simple text editor or word processor and means that teachers may alter existing text (for example, translate into different languages), add new text or assignments, and in effect tailor the program to their specific needs.

Some of these programs provide clinical data and feature case histories of typical clinical disorders. These sections are highly interactive and require students to interpret clinical test results and to make appropriate diagnoses. They also cover the physiological basis of the laboratory tests.

Interactive tutorials – designed to support lectures or seminars. Text, graphics, and features such as animation and hypertext are combined with student-centered tasks and problems to produce quality learning aids suitable for independent study. These programs require a slightly higher specification machine (minimum 386 PC, 33 MHz, VGA graphics, and Microsoft Windows 3.1).

Programs include *Introduction to Endocrinology, Rat Blood Pressure, Respiratory Pharmacology, Intestinal Motility, PK-SIMS* (pharmakokinetic simulations), *Drug Disposition Tutorials, Pharmacology of Inflammation, Neuromuscular Pharmacology, Langendorff Heart, Finkelman* (simulation of the Finkelman preparation to teach pharmacology), *Guinea Pig Ileum, Cat Nictitating Membrane, Epilepsy Tutorial, The Heart Tutorial, The Circulation Tutorial, Human Nervous System, Asthma, Cellular Respiration, Clinical Aspects of Pain, Squid Axon, Nerve Biophysics Tutorial, Blood Coagulation, Chest Clinic, Intestinal Absorption, Blood Physiology, Frog Heart, The Electrocardiogram, Exercise Physiology, Frog Skin* (to study membrane transport of ions), *Nerve Physiology,* and *Muscle Physiology*.

For more information, contact David Dewhurst, Ph.D., Sheffield Bioscience Programs, 5 Woodlands Green, North Yorkshire HG2 8QD, United Kingdom, phone: +44 (0) 1423 888514, e-mail: d.dewhurst@lmu.ac.uk

- **Alternatives in Veterinary Education Newsletter**

This newsletter, published by the Association of Veterinarians for Animal Rights (AVAR), contains valuable information for those looking for sources of alternatives in veterinary, medical, or graduate education. The newsletter also spotlights veterinary school programs that have implemented alternatives into their traditional curriculum (for example, Issue III 1996 focused on the School of Veterinary Medicine at Washington State University). For more information or to subscribe, contact AVAR, P.O. Box 208, Davis, CA 95617-0208 USA, phone: (916) 759-8106, fax: (916) 759-8116, e-mail: AVAR@igc.apc.org, World Wide Web: http://www.envirolink.org/arrs/avar/avar_www.htm

- **European Collection of Cell Cultures and the Immunoclone Database**

The European Collection of Cell Cultures (ECACC) is based at the Centre for Applied Microbiology and Research (CAMR). CAMR is a Special Health Authority of the [United Kingdom] Department of Health. The collection is supported from a combination of sources, the UK Research Council, the European Community, the World Health Organization, and revenue from sales and the provision of technical services.

All distribution stocks of cell lines are tested for viability and for absence of mycoplasma. It is ECACC policy not to distribute mycoplasma infected material.

Cell lines designated "RCD" are part of the Research Council's Collection – ampules from this collection are free of charge to UK nonprofit organizations; however, all requests are obliged to pay transportation costs. If a growing culture is required then a flask fee is charged.

Other collection deposits are available at ECACC's current standard charges.

Immunoclone Database

A European collaborative venture has formed a unique database specifically designed for researchers interested in immunoclones, such as monoclonal antibodies and T-cell receptors. The database provides a facility for on-line searches of specific antigens or immunoclones.

U.S. orders – Import permit requirements

It is an obligatory requirement for the importation of cell lines or their products into the United States that an application be made for a veterinary permit from the U.S. Department of Agriculture prior to shipment. The procedure for obtaining a permit is as follows:

1. Forms VS 16-3 and VS 16-7 are requested by recipient of the cells from: USDA, APHIS, VS Import-Export Center, 4700 River Road, Unit 40, Riverdale, MD 20731 USA, phone: (301) 734-3277, fax: (301) 734-8226. The recipient must complete the forms and return them to the above address. Faxed copies are accepted. A copy of the certificate of origin for the serum used in the culture of cells should also be included. This can be supplied by the ECACC.

2. The Department will then issue a permit (VS form 16-16A) to the recipient or directly to the ECACC if requested at the time of application.

For more information , contact the ECACC, Centre for Applied Microbiology and Research, Salisbury, Wilts SP4 OJG, United Kingdom, phone: +44 1980 612 512, fax: +44 1980 611 315, e-mail: ecacc@ecacc.demon.co.uk or contact the Centre Europeen de Recherche et de Developpement en Information et Communication Scientifiques (CERDIC), BP 295, 06905 Sophia Antipolis, France, phone/ fax : (33) 93 95 86 38. Orders can be placed by fax or post, they are unable to accept orders by telephone.

- **Primate Enrichment Listserv Discussion Group**

David Seelig at Yale University has established an e-mail list devoted to discussion of topics in primate enrichment at biomedical and behavioral research laboratories. Individuals from zoological parks are also invited to participate. The list currently has 250 subscribers. If you are interested in participating, please send a message to david.seelig@yale.edu or visit the Primate Enrichment Forum website at http://pantheon.yale.edu/~seelig/pef/

AVAILABLE ON THE WORLD WIDE WEB

- **Animal Alternatives**

http://www.vetmed.ucdavis/edu/Animal_Alternatives/main.htm

The home page of the UC Center for Animal Alternatives at the University of California at Davis School of Veterinary Medicine. The center places emphasis on distributing information about models, computer simulations, and other animal alternatives in education through every level of public and private education. It also seeks to provide investigators who use animals with information on the most current methods for improving all aspects of animal care.

- **Centrum Welzijn Dieren-Animal Welfare Center, Utrecht University**

http://www.dgk.ruu.nl/algemeen_bijzondere_centra_icwd.htm

The center's goal is to promote and initiate research into animal welfare with a research emphasis on the neurophysiological basis for an animal's welfare. The page is in Dutch and English.

- **Canadian Council on Animal Care/Conseil canadiene de protection des animaux**

http://www.ccac.ca

The website provides information on the primary goals of the CCAC: assessment, education, and development of animal care guidelines. On-line requisition of many of their publications (*Guide to the Care and Use of Experimental Animals, Orientation Package for Animal Care Committee Members, Animal Use Protocol Review, etc.)* is available. The page is in English and French.

- **The Shape of Enrichment**

http://www.enrichment.org

You've read the newsletter, now check out their website! Provides general information about subscribing to their newsletter, list of enrichment videos available for loan (includes sample clips of some videos), enrichment conference and workshop announcements, publications, etc. Focuses on zoo animals, but many ideas may apply to other animals.

- **State Statutes on the Internet**

http://www.law.cornell.edu/topics/state_statutes.html

Provides access to State statutes organized by topic. Topics include agriculture, fish and game, natural resources, etc. Many animal cruelty statutes can be found under agriculture.

- **Blood collection in mice using the saphenous vein – An alternative to retro-orbital collection**

http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.htmlP

hotographs and text illustrate this blood-collection method developed by Annelise Hem and Per Solberg at the Laboratory Animal Centre of the Norwegian Institute of Public Health. The method as shown is performed at the University of Bergen and shows modifications developed at the vivarium of the university.

- **Teaching materials in laboratory animal science**

http://oslovet.veths.no/teaching/materials.html

These pages are an initial attempt to collect links to web pages and other sources containing teaching texts on subjects related to pure laboratory animal science. These include ethics, legislation, comparative biology, anatomy, pharmacol-

ogy, physiology, genetics, anesthesia, analgesia, euthanasia, microbiology, environmental factors, experimental design, and techniques. Submission of URLs may be made to Adrian Smith, D.V.M., at e-mail: adrian.smith@veths.no

- **Virtual tour of a laboratory animal unit**
  http://oslovet.veths.no/omvisning/omviseng.html

Never seen the inside of an animal facility? Then check out the animal facility at the Norwegian College of Veterinary Medicine. This excellent virtual tour illustrates environmental enrichment techniques for rodents and mink and group housing of rabbits and provides information on alternatives to the use of animals.

- **Amphibian Information Center**
  http://monitoring2.pwrc.nbs.gov/amphibs/

This searchable database is being developed by the Patuxent Wildlife Research Center, the U.S. National Park Service, and the U.S. Environmental Protection Agency. ■

Humane Society of the United States Highlights Alternatives to Animal Testing

The Humane Society of the United States (HSUS) has presented the 7th annual Russell and Burch Award to Dr. Horst Spielmann of the German National Center for the Documentation and Evaluation of Alternatives to Animal Experiments. The award includes a $5,000 prize.

"The Russell and Burch Award recognizes scientists who have made outstanding contributions to the advancement of alternatives to using animals (Russell and Burch first initiated an alternatives approach to animal use)," says Dr. Martin Stephens, HSUS vice president for animal research issues. "Dr. Spielmann joins an illustrious group of scientists receiving this award, including Johns Hopkins University's Dr. Alan Goldberg. The award presents a unique opportunity for the nation's largest animal protection organization to recognize the important work of scientists who improve the lives of animals used in research, testing and education."

The HSUS established the Russell and Burch award to encourage scientists to pursue new avenues in the alternatives arena. Alternatives to animals in research, testing, and education include replacing animals with substitute methods, reducing the number of animal subjects and refining experiments to diminish pain and suffering.

Dr. Spielmann is a member of the management teams of several validation studies on alternatives, including those in the fields of phototoxicity, embryotoxicity, eye irritation, and skin irritation.

According to Dr. Andrew Rowan, HSUS senior vice president, "Dr. Spielmann has been a leader in the alternatives field not only in his native Germany, but throughout the European Union and beyond. His diverse work on alternatives includes conducting research, managing multilaboratory projects, and serving as an advisor to the European Centre for the Validation of Alternative Methods and other organizations." ■

National Animal Poison Control Center (NAPCC)

This 24-hour emergency service, sponsored by the American Society for the Prevention of Cruelty to Animals (ASPCA), is the first and only national animal-oriented poison information center in the United States. Since 1978, it has provided advice to animal owners and conferred with veterinarians about poisoning exposures. The center's telephones are answered by licensed veterinarians and board-certified veterinary toxicologists.

The center is staffed by veterinary health professionals who are familiar with different species' response to poisons and treatment protocols. At their fingertips, they have a wide range of information specific to animal poisoning, including an extensive collection of individual cases – more than 250,000–involving pesticide, drug, plant, metal, and other exposures in companion, wild, and food-producing animals. This specialized information lets the experienced ASPCA National Animal Poison Control Center staff make specific, accurate recommendations for your animals, as opposed to the generalized poison information available from human poison control centers.

Depending on which option is chosen, the charge is $20 for the first 5 minutes, then $2.95/minute thereafter when using the 900 number, or $30 per case (Visa, MasterCard, Discover or American Express **only**) when using the 800 or 888 number. With the 800 or 888 access **only**, the Center will make as many follow-up calls as necessary. Through the ASPCA/NAPCC Animal Product Safety Service, various veterinary medicine and chemical product manufacturers list a toll-free 800 telephone number on their products and help to underwrite cases involving those products.

If an animal has been poisoned, immediately call the ASPCA National Animal Poison Control Center. Be ready to provide: 1)Your name, address, and telephone number; 2)The substance your animal has been exposed to, if known; 3)Information concerning the exposure, including the amount of substance, the time since exposure to the substance, etc. *(It is very important to provide accurate information on the substance. If the substance is listed in the Animal Product Safety Service, the consultation is at no cost to the caller.)*; 4)The species, breed, age, sex, weight and number of animals involved; and 5)The problems your animal is experiencing.

If you are unable to access the 900 number, call your telephone company for assistance, or use the 800 or 888 number. When the 800 or 888 number is used, your credit card number will be required in addition to the above information.

The ASPCA National Animal Poison Control Center offers manufacturers of veterinary, agricultural, and chemical products an extensive animal product safety program. The program provides a toll-free telephone number which can be printed on product labels and literature, assisting owners and veterinarians with questions or suspected poisonings. The program also offers management of case records, compilation of quarterly case reports and consultations with the manufacturer's professional staff to improve product safety. Additional services are available to tailor an animal product safety program to meet each manufacturer's needs.

For additional flyers or more information about the Center's various services, please contact:

ASPCA National Animal Poison Control Center, 1717 South Philo Road, Suite #36, Urbana, IL 61802 or The ASPCA, 424 East 92nd Street, New York, NY 10128-6804, phone: (212) 876-7700, extension 4656. ■

Grants...

- **The Alternatives Research and Development Foundation (ARDF)**

As part of its continuing efforts to encourage the use of alternatives to traditional uses of laboratory animals in basic research, testing, and education, the ARDF is soliciting research proposals to develop such methods. Funding of up to $40,000 each is available to support individual projects at U.S. universities and institutions. Deadline for applications is April 30, 1998, with recipients to be announced on July 15, 1998. For further information and application instructions, contact The Alternatives Research and Development Foundation, 14280 Golf View Drive, Eden Prairie, MN 55346, fax: (612) 949-2619.

- **AAZK Zoo Keeper Grant in Research**

The American Association of Zoo Keepers (AAZK) has developed the Zoo Keeper Grant in noninvasive research to promote and support keeper and aquarist efforts in behavioral research. The $750 research grant is for the benefit of North American zoological research efforts. The deadline for applications is March 1, 1998. AAZK members in good standing should direct their inquiries to: Farshid Mehrdadfar, Chair, AAZK Research Grant Committee, Disney's Animal Kingdom, P.O. Box 10000, Lake Buena Vista, FL 32830-1000, e-mail: farshid@sprynrt.com

- **The Pfizer/LASA Animal Welfare Research Award Fund**
  Call for Applications

This fund was established in 1993 by means of an annual grant made by Pfizer Central Research in the United Kingdom to the Laboratory Animal Science Association (LASA). Pfizer and LASA intend the fund to be used to support research that is concerned with any aspect of animal welfare associated with laboratory science, particularly that concerned with the 3Rs of reduction, refinement, and replacement.

Applications are now invited for awards to be made in early 1998. Further details and application forms are available from the LASA Secretariat (address below) and should be submitted by February 28, 1998. Both Pfizer and LASA are keen to support sound research projects that will further animal welfare in laboratory science, and you are encouraged to apply to the Fund. LASA Secretariat, P.O. Box 3993, Tamworth, Staffordshire B78 3QU, UK, phone/fax: 01827 260036, e-mail: lasa@globalnet.co.uk

- **Foundation Research 3R-Stiftung Forschung 3R-Fondation Recherches 3R**

The Foundation Research 3R is calling for research proposals that will advance the 3Rs concept of alternatives–reduction in the number of animals used, refinement of painful techniques, and replacement with nonanimal methods. No official application form is necessary. Research will be evaluated based on its concordance with the 3R program and its scientific and public impact. The deadline for submission of proposals is March 1, 1998. For additional information, contact Ronald Greber at phone: +41 31 722 08 31, fax: +41 31 722 08 34, e-mail: ronald.greber.3r@bluewin.ch

- **Sources of Support for Research on Alternatives**

A directory of 16 organizations that fund research into methods that will reduce, refine, or replace the use of animals in research can be found at http://www.uiowa.edu/~vpr/research/animalt.htm

The listing, maintained by the University of Iowa, was last updated in 1996, but contact information is correct.

- **NonProfit Gateway**

The NonProfit Gateway is a network of links to Federal Government information and services including funding opportunities. It is available at http://www.nonprofit.gov The page includes a search box where you can search more than 300,000 Government web pages. ■

Center for Alternatives to Animal Testing Call for Research Proposals

The Johns Hopkins Center for Alternatives to Animal Testing (CAAT) is soliciting proposals for the 1998-99 grant period. The proposed research should provide fundamental knowledge needed to develop replacement alternative tests for safety/hazard evaluation, risk assessment and efficacy of commercial products.

We encourage the investigation of in vitro approaches to evaluating cellular and target organ toxicity. Some examples are: developing new cell culture systems, applying current testing methodology to human cells/cell lines, and designing new, mechanistic, state of the art methods that may utilize cultured cells, computer technology (e.g. structure activity relationships), or any other system applicable to toxicity/efficacy evaluation. At the present time, CAAT does not fund projects relating to carcinogenically or mutagenicity, or those not focused on developing testing strategies. The maximum grant award for this period will be $20,000.

Applications must be placed on a CAAT Preproposal Abstract Form (98-99). These forms are available in January, 1998 from Gloria Mahlstedt at CAAT, 111 Marketplace, Suite 840, Baltimore, MD 21202-6709, Tel. (410)223-1693, Fax (410)223-1603, E-Mail gloria@caat.spharbor.jhu.edu

Deadline for Submission Of Preproposal Abstracts: March 16, 1998.

"Meeting the Information Requirements of the Animal Welfare Act"

The Animal Welfare Information Center (AWIC) of the U.S. Department of Agriculture, National Agricultural Library (NAL) has developed a 2-day workshop for individuals who are responsible for providing information to meet the requirements of the Animal Welfare Act. Representatives from NIH, Office of Protection from Research Risks, and USDA's APHIS, Animal Care will be available for questions and answers . The workshop will be held at NAL in Beltsville, Maryland.

The act requires that investigators provide Institutional Animal Care and Use Committees (IACUC) with documentation demonstrating that a thorough literature search was conducted regarding alternatives. An alternative is any procedure that results in the reduction in the numbers of animals used, refinement of techniques, or replacement of animals.

The objectives of the workshop are to provide:

- an overview of the Animal Welfare Act and the information requirements of the act.
- a review of the alternatives concept.
- a comprehensive introduction to NAL, AWIC, and other organizations.
- instruction on the use of existing information databases/networks.
- online database searching experience.

This workshop is targeted for principal investigators, members of IACUC's, information providers, administrators of animal use programs, and veterinarians. All participants will receive a resource manual.

The workshops will be held on March 5-6, June 24-25, and October 28-29, 1998. The workshop will be limited to 20 people. There is no fee for the workshop.

For more information, contact AWIC at phone: (301) 504-6212, fax: (301) 504-7125, or e-mail: awic@nal.usda.gov, or write to: Animal Welfare Information Center, U.S. Department of Agriculture, National Agricultural Library, 10301 Baltimore Avenue, Beltsville, MD 20705-2351 ■

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